

ADDENDUM

Dr. M. C. Terry (PROCEEDINGS, 1935, **33**, 14) overlooked a report of the same experiment and result by R. A. A. O'Meara (*J. Path. and Bact.*, 1935, **37**, 166) and wishes to acknowledge priority.

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8419 P*

Respiratory Behavior of the Propionic Acid Bacteria.

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Redox potential data obtained with resting cells of propionic acid bacteria, using succinic, fumaric, or malic acids as hydrogen donors, with various hydrogen acceptors show the following results. Succinic acid acts as a donator to methylene blue, NaNO_3 , and o-chlorophenol-indophenol. Fumaric acid may donate hydrogen to a less degree to methylene blue and o-chlorophenol-indophenol, while malic acid shows only a slight donation to these acceptors. Respiration experiments using the Barcroft-Warburg technique show marked O_2 -uptake with buffered suspensions of propionic acid bacteria when succinic, lactic, and pyruvic acids are used as substrates; much less with malic and fumaric acids. In an atmosphere of nitrogen, CO_2 is evolved in relatively large quantities from succinic, lactic, and

* P represents a preliminary, C a complete manuscript.

pyruvic acids, and in smaller amounts from fumaric and malic acids. From these results it is concluded that the conversion of succinic acid to propionic acid proposed by Wood, Stone and Werkman¹ probably takes place by direct decarboxylation and not by way of fumaric, malic, and oxalacetic acids as intermediates.

8420 C

The Bill of the Sparrow as an Indicator for the Male Sex Hormone. I. Sensitivity.*

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Research on the male sex hormone has long been hampered by the lack of indicators which at the same time are sufficiently sensitive, inexpensive and simple from a technical viewpoint. Keck¹ reported the fact that pigmentation of the male sparrow bill depends on the presence of the male sex hormone. Using a preparation which had been extracted in our laboratory from male urine and standardized by the rat seminal vesicle test, he concluded that in the bill of male castrates as little as 1/15 of a daily rat unit gives a distinct reaction by the eighth day.

Since the sparrow can be kept with little expense even in small laboratories and the color change is so distinct and directly observable, it seemed worthwhile to investigate the availability of this reaction as a hormone indicator. We here report on a quantitative study, comparing the sparrow unit with the capon and the rat units.

Castration causing permanent depigmentation is easily performed in the sparrow. Even in the unoperated male, the bill is light brown outside of the breeding season, as is the bill of the female, with few exceptions, during the whole year. It would considerably facilitate the use of the test if such unoperated specimens should react the same way as the male castrate. This possibility has been thoroughly investigated as well as the question whether the duration of castration has some influence on the color response.

¹ Wood, H. G., Stone, R. W., and Werkman, C. H., *J. Bact.*, 1935, **29**, 84.

* This investigation was supported by grants from the National Research Council, Committee for Research in Problems of Sex.

¹ Keck, W. N., *J. Exp. Zool.*, 1934, **67**, 315.

The writer is greatly indebted to Professor F. C. Koch of the University of Chicago, who readily consented to collaborate with us by the supply of 3 samples of standardized male hormone preparations obtained from human urine, namely:

Sample 1—UVC4: 45 BU/cc. (approximately 0.1 cc. = 0.75 r.u.)
 " 2—UVC5: 15 BU/cc. (" " 0.1 " = 0.25 r.u.)
 " 3—UVC6: 6 BU/cc. (" " 0.1 " = 0.1 r.u.)

According to Koch, the strength of these preparations was standardized directly in "bird units (BU)" (we would prefer the terms *comb* or *capon* unit) while the rat units (r.u.) given represent only a calculated estimation. Table I contains the results obtained by the injection of this material into castrate and non-castrate males and females. Since the experiments were carried out in the month of December and with birds kept indoors for several months, all bills were of the very light brown color indicating absence of male hormone at the time when the injections were started.

TABLE I.

Bird No.	Sex	Time Castrated, mo.	Daily Injection, .1 cc. of prep.	Reaction after		
				5 days	10 days	20 days
450	♂	3.5	UVC4	III	IV	*
434	"	3.5	"	II	IV	*
500	♀	3.5	"	III	IV	IV
440	"	3.5	"	II	III	*
4	♂	—	UVC5	II	III	IV
16	"	—	"	II	III	IV
17	♀	—	"	I	III	III
18	"	—	"	I	II	II
425	♂	18	UVC6	II	III	IV
463	"	3.5	"	II	III	IV
497	"	3.5	"	III	IV	IV
386	♀	3.5	"	0	II	III
429	"	3.5	"	0	III	*

0, no reaction; I, darkened brown; II, light blue; III, dark blue; IV, black; *, killed.

The data presented in the table show that the pigment reaction appears in both sexes, castrates as well as non-castrates. Two of the females (500, 386) were less than one year old but responded as well as the adult ones. However, the present experiment confirms our general experience that the bill of the female sparrow is slower in response and does not give as complete a reaction as the male bill. Some regions, especially the under surface of the lower bill, always stay brown, due to the lack of melanophores. It is truly remarkable that the female bill responds at all in view of the

fact that in the normal life cycle, pigmentation of such extent never occurs. The histological study of the reaction which will be presented in a following paper provides a full explanation of this situation.

Normal males and females of the quiescent sex period (birds No. 4, 16, 17, 18) respond nearly as well as castrates. The sex glands are not activated by the hormone injections and it is not probable that they contribute hormonally in any way to the reaction. Considering, however, that increasing illumination might easily stimulate the testes and thus bring about a pigmentation of the bill interfering with the testing procedure, it seems advisable to make use only of castrate males in the quantitative assay of hormone preparations.

With the exception of one, the castrates listed in the table were injected $3\frac{1}{2}$ months after the operation. Male 425, which had been castrated for 18 months reacted nearly as readily and fully as completely. Keck in his above quoted work had used 14 castrates 6 months after the operation. In these as in other series of experiments, we have never observed a distinct change in sensitivity with the age of castration.

As a rule 10 daily injections give a full color reaction. Discontinuation of treatments at this time is still followed by further darkening of the bill color, due to the movement of the pigment layer toward the surface of the bill. About 20 days after the first injection the pigment reaches the surface. In the third week after the last injection it begins to wear off, the unpigmented layers appearing first as a white ring around the base of the bill. Five weeks after the last administration of the hormone the bill has practically lost all its pigment again.

It is of importance that the reaction in its first stage is not merely quantitative but consists in a characteristic change from light brown to blue. The blue tint is a physical effect, due to the deposit of melanin granules below the cornified strata of the epidermis.

The outcome of the experimental series with Professor Koch's standardized preparations proves the correctness of our previous evaluation of the sparrow unit (SU) as the equivalent of one-tenth (or even less) of the rat unit and approximately one-half of the Chicago capon unit (BU).

8421 C

Effect of Vitamins B and G on Coccidian Infection in the Rat.

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The writers¹ recently reported their discovery that the number of oocysts eliminated by rats infected with *Eimeria miyairii* after the host had been for 9 days on a diet deficient in both vitamins B and G was only one-fourth to one-fifth that in the reference series which received a so-called normal diet. The present study was made to determine whether the observed effect was due to the absence of one or both of the vitamins.

The basal ration was as follows: Extracted casein, 18%; normal (Harris') salt mixture, 3.7%; butter, 4.0%; cod liver oil, 1.0%; bacto-agar, 2.0%; beet sugar, 71.3%. The vitamin-B deficient ration consisted of the basal ration with 12 of the 71.3 parts of sugar replaced with powdered (Fleischmann's) yeast that had been autoclaved for more than 3 hours at 120°C. and dried. The control or reference diet for the rats on the vitamin-B deficient diet consisted of the basal ration with 10 parts of the sugar replaced with the same yeast unautoclaved. The vitamin-G deficient ration consisted of the basal ration plus 1 cc. of wheat germ extract. The latter was equivalent to 2 gm. of the wheat germ. Its preparation will be described in a later paper. The reference diet for the rats receiving the vitamin-G deficient ration consisted of the basal diet with 10 parts of sugar replaced with autoclaved yeast and, likewise, one cc. of wheat germ extract each day.

Experiment I. On October 31 nine rats were started on the vitamin-B deficient ration. One November 14, 15, 16 and 18 each rat received about 2100 sporulated oocysts of *Eimeria miyairii*. The reference series was started on the control diet the same day, and infected on the same days and in the same way as the deficient series. The oocysts appeared in the feces from November 21 to November 27, and were collected in pans containing a disinfectant placed beneath the hardware cloth bottoms of the individual rat cages. The deficient series averaged 69 gm. in weight on October 31, 88 gm. on Nov. 14, 70 gm. on Nov. 21, and 102 gm. on Nov. 28. The increase in weight between November 21 and Nov. 28 is due to the

¹ Becker, E. R., and Morehouse, N. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 114.

fact that the animals were placed on Steenbock's growing ration beginning Nov. 24 in order to flush from the intestine any oocysts that might be retained on account of intestinal immobility resulting from the vitamin deficiency. The reference series averaged 66 gm. on Oct. 31, 102 gm. on Nov. 14, 114 gm. on Nov. 21, and 135 gm. on Nov. 28. These rats were also placed on Steenbock's ration Nov. 24. All animals survived the experiment except one vitamin-deficient which died from an unknown cause on Nov. 24.

The deficient series eliminated, respectively, the following numbers of oocysts expressed in millions: 166, 218, 207, 279, 204, 261, 262, 328; mean, 240.63. The reference series: 255, 257, 233, 242, 222, 151, 247, 245, 269; mean, 235.67. Since the means are so close, there is no reason to believe the vitamin-B deficiency affected the infection in any way so far as the number of oocysts that were developed is concerned.

Experiment II. On November 15 nine rats were started on the vitamin-G deficient diet, and 4 more on Nov. 18. The 9 were infected on Nov. 27, 28, 29, and Dec. 1 with doses of 2100 oocysts, the 4 on Nov. 29, 30, Dec. 1 and 3 with the same dosage. The oocysts were collected from both sets of rats as described above from the seventh to the thirteenth day, inclusive, after the date of the first inoculation of each set. The deficient rats averaged 78 gm. when placed on the special diet, 83 gm. at the time of inoculation, 82 gm. 10 days after inoculation, when they were placed on Steenbock's growing ration in order to flush possibly retained oocysts from the intestine, and 102 gm. on the fourth day thereafter. The controls averaged 79 gm. when placed on the reference diet Nov. 15, 106 gm. when given the first infective dose on Nov. 27, 122 gm. 10 days later, when they also were placed on the Steenbock's ration, and 130 gm. the fourth day thereafter. All rats survived except one vitamin-G deficient which died on the tenth day after the first inoculating dose.

The vitamin-G deficient series eliminated by individuals the following numbers of oocysts expressed in millions: 119, 36, 89, 131, 61, 82, 69, 51, 116, 37, 58, 30; mean, 73.25. The reference series: 322, 132, 192, 337, 172, 412, 116, 196, 124; mean, 222.55. The mean for the reference series is approximately 3 times that for the deficient series.

Experiment III. This experiment was done in connection with Experiment II, and the controls are the same in both cases. Four rats were placed on the basal ration deficient in both vitamins B and G on Nov. 18. The treatment was the same as in the case of the set

of 4 rats deficient in vitamin G described in Experiment II, except that the wheat germ extract was not fed. The rats lost weight slowly on the diet, from an initial 77 gm. mean on Nov. 18 to 75 gm. on Dec. 19, when they were placed on the Steenbock ration, and 4 days later averaged 92 gm. The counts in millions of oocysts were as follows: 62, 65, 12, 47; mean, 46.5. This mean is to be compared with that of 222.55 in the controls on the complete diet, and 73.25 in the vitamin-G deficient.

Experiment III confirms our previous finding that a host on a diet deficient in both vitamins B and G is not as suitable a medium for the development of a coccidium as one receiving an adequate diet. The question arises, is this difference due to the combination of vitamin deficiencies or to one or other of the vitamins? Experiment I appears to show that it is not due to vitamin B. The three to one ratio of the mean oocyst count for the infections in the reference or normal series to the mean oocyst count for the vitamin-G deficient series indicates that all or most of the difference is attributable to vitamin G. It is true that the ratio is not so high as the previously reported 4.67 between the counts for the normal and both vitamins B and G deficient animals, but it is to be considered that the alcoholic extract of wheat germ used as a source of vitamin B probably contained some vitamin G. According to Feaster,² an extract prepared similarly to ours contained about one-fifth as much vitamin G as B. Hence it is apparent that the animals receiving the extract as a source of vitamin B were receiving considerably more vitamin G than those in the series receiving no material as a source of either vitamin G or B.

It is to be made clear that the work is in the early experimental stage, and that the results here reported are not to be construed as indicating practical application of vitamin-G deficiency as a control for coccidiosis. In the first place, the effects of upsetting the nutritional balance of an animal might be worse than the infection. In the second place, we have not yet determined whether the lower parasite level on the vitamin-G deficient diet sufficiently counterbalances the lowered vitality of the host to create a differential in favor of the host. The findings, however, open up a number of problems relating to the host-parasite balance in coccidiosis.

This investigation was supported in part by a grant from the Industrial Science Research funds of the Iowa State College for the study of host-parasite relationships in coccidiosis. Special thanks

² Feaster, J. F., Doctoral Thesis, Iowa State College Library.

are due to Dr. V. E. Nelson and Mrs. Gladys Timson Stevenson of Iowa State College for valuable information relative to the preparation of the diets.

8422 P

Preservation of Fertility in Male and Female Rats on a
Supplemented Milk Diet.

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From the Laboratories of Physiological Chemistry, Iowa State College.

This work is the outgrowth of the studies made by the authors on the relation of iron and copper to nutritional anemia and hemoglobin synthesis. It is well known that cow's milk is inadequate for the normal maintenance of the rat. Additions of copper and iron salts to an exclusive milk diet protect animals from nutritional anemia and produce an apparent well being in the animals of the first generation from the standpoint of hemoglobin formation, growth, and reproduction. Underhill, Orten, Mugrage, and Lewis¹ have observed no abnormalities in the organs of rats fed milk, copper, and iron for periods of more than a year. Waddell² reported sterility in males of the first generation receiving a milk, copper, and iron diet. The sterility was marked by a progressive testicular degeneration together with an absence of motile sperm in the seminal fluid. Ferric chloride, when added to the ration, was found to produce symptoms of sterility as early as 10 weeks after administration. Keil, Keil, and Nelson³ found the animals on a milk, copper, and iron ration grow more slowly than those on a good stock diet. Reproduction occurred in animals of the first generation, but the second generation did not reproduce. Our results on second generation rats, together with the findings of Waddell, led us to investigate both males and females of the first and second generations in order to ascertain whether or not the sterility on this diet could be ascribed to degeneration of sex organs.

All of the animals (rats) were fed whole milk obtained from Holstein cows in the dairy herd at Iowa State College. The milk was collected directly into glass jugs, in order to avoid contamina-

¹ Underhill, F. A., Orten, J. M., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **99**, 469.

² Waddell, J., *J. Nutr.*, 1931, **4**, 67.

³ Keil, H. L., Keil, H. H., and Nelson, V. E., *Am. J. Physiol.*, 1934, **108**, 215.

tion with inorganic substances. Purified copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and iron as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added to the fresh milk daily. Copper and iron salts were examined spectrographically, in order to insure a high degree of purity. Hemoglobin was determined by the Newcomer method. The animals were bled by the tail.

The trimmed testes from the males were weighed and placed in Bouin's fixing solution prior to histological sectioning. The epididymis was cut and semen squeezed into a drop of Ringer's solution on a microscopic slide, which was immediately examined for motile sperm. The males were also subjected to mating tests with unbred females from the stock colony. Many females of the second generation on a diet of milk, copper, and iron were tested for sterility at the end of an experiment by placing them with mature males from the stock diet. These animals were given the milk, copper, and iron diet. Other females were tested in a similar way, except that all of the rats were given stock ration during the period of breeding. The ovaries of the female rats were trimmed and weighed; and they were then fixed in Bouin's solution and examined histologically.

Galvanized iron wire cages placed on glass funnels were employed for metabolism cages in the studies on the composition of the urine. Analyses of urine were made on 3 groups of animals. One group had been fed milk, copper, and iron from the time of weaning; a second lot was depleted of its copper and iron stores by an exclusive milk diet until a state of anemia existed; and a third group was given the stock colony ration. The following determinations were performed on the urine: volume, specific gravity, total nitrogen, urea, ammonia, uric acid, creatine, and creatinine. The results of the experiments may be summarized as follows:

No sterility or degeneration of the testes was observed in male albino rats maintained on milk, supplemented with various amounts of purified iron as FeCl_3 together with copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The testicular weights from both first and second generation males on the above ration compared favorably with those obtained on the stock diet; motile sperm were found in all cases, and mating tests were positive.

The average weights of paired ovaries from female rats reared on a diet consisting of milk supplemented with pure iron as FeCl_3 along with copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is slightly lower than that of controls on a stock diet. This apparent abnormality did not denote sterility, since normal litters have been obtained in the second generation

after the rats were given a stock ration in lieu of the milk, copper, and iron diet.

A nitrogen partition in the urine of 3 groups of female rats fed stock ration, milk, and milk plus copper and iron, respectively, indicates that the addition of copper and iron to the milk diet affects the excretion of nitrogen bodies, particularly ammonia, creatine, and creatinine.

8423 P

Effect of Methods of Preparation upon Fermentative Activity of Yeast Zymin.

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Stavely, Christensen and Fulmer^{1, 2, 3, 4} presented data on the effect of several electrolytes and of ethanol upon the fermentative activity and phosphate content of yeast zymin. The zymin was prepared from brewers' yeast (furnished by Anheuser-Busch of St. Louis) by the procedure outlined by Harden.⁵ Five hundred grams of yeast and 3 liters of acetone were stirred together for 10 minutes and filtered on a Buchner funnel. The mass was then mixed with one liter of acetone for 2 minutes and again filtered. The residue was coarsely powdered, stirred with 250 cc. of ether for 3 minutes, filtered, and spread out on paper in a thin layer. After standing for one hour at room temperature it was dried for 24 hours at 40-45°.

The present communication deals with data on several factors as they affect the activity of the zymin preparation. The apparatus of Harden, Thompson and Young,⁶ with some modification, was employed in the determination of the evolution of carbon dioxide. The

¹ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 877.

² Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 771.

³ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 785.

⁴ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 791.

⁵ Harden, A., *Alcoholic Fermentation*, p. 39. Longmans, Green and Co.

⁶ Harden, A., Thompson, J., and Young, W. J., *Biol. Chem. J.*, 1910, **5**, 230.

inorganic phosphate was determined colorimetrically by the revised procedure of Kuttner and Lichtenstein.⁷ Detailed procedure and technique are given in reference 2.

It was noted by Stavely, Christensen and Fulmer² that the fermentative activity of the zymin increased with the time of storage, in the refrigerator, of the yeast from which it was made. The increase was paralleled by increase in free inorganic phosphate and in the rate of esterification of the phosphate in the presence of dextrose. In Tables I and II are given more detailed data with reference to the points noted above. The yeast was stored at 5°. The reaction mixture consisted of 5 gm. zymin, 1 gm. dextrose, 1 cc. toluene, and 25 cc. of water. The fermentative activity increased up to 14 days' storage of the yeast, but at 20 days the preparation was inactive. This increase in fermentative ability is seen to be correlated with an increased rate of esterification of the inorganic phosphate

TABLE I.
Effect of Storage on CO₂ Evolution by Zymin (Mg. of CO₂ Evolved).

No. Days Storage Time:	0	1	3	5	8	14	20
10	0.0	3.2	1.1	0.0	0.0	0.0	
20	3.3	9.1	15.1	7.4	3.2	5.7	
30	9.1	23.8	28.8	32.5	20.1	16.5	
40	12.2	34.7	42.2	45.2	35.6	30.5	Inactive
50	18.2	46.3	48.8	51.9	46.7	43.4	
60	23.4	52.3	54.1	57.2	55.7	55.6	
70	29.7	56.5	58.3	61.5	60.4	64.5	
90	43.5	66.0	66.4	70.5	70.8	74.3	

TABLE II.
Effect of Storage on Phosphate Esterifying Ability (Mg. Inorganic P per cc.).

No. Days Storage Time:	0	1	3	5	8	14	20
10	0.65	0.62	0.78	0.85	1.08	1.25	1.15
30	0.36	0.32	0.22	0.26	0.72	0.84	1.09
60	0.30	0.18	0.19	0.19	0.18	0.23	1.10
90	0.32	0.24	0.19	0.17	0.15	0.18	—
Change in P (10-90 min.)	0.33	0.38	0.59	0.68	0.93	1.07	—

In the procedure of Harden the material is dried at 40-45° for 24 hours. Two zymin preparations were made, one by the usual procedure, drying at 45° and the other by drying in a vacuum. Such preparations were made from yeast that had been stored 0 and 3 days at 5°. The fermentative activities of these preparations are given in Table III. It is evident that the vacuum drying gives a zymin which shows a greater initial evolution of carbon dioxide and

⁷ Kuttner, J., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

TABLE III.
Effect of Vacuum Drying on CO₂ Evolution by Zymin (Mg. CO₂ Evolved). Zymin 1 had been stored 0 days and Zymin 2 for 3 days.

Time (min.)	Zymin 1		Zymin 2	
	Control 45°	Vacuum Dried	Control 45°	Vacuum Dried
10	0.0	3.5	1.1	8.4
20	3.3	12.3	15.1	36.6
30	9.1	20.8	28.8	48.8
40	12.2	27.8	42.2	57.2
50	18.2	33.2	48.8	61.8
60	23.4	39.8	54.1	69.2
70	29.7	47.5	58.3	74.7
90	43.5	60.9	66.4	86.1

a more rapid decrease in rate after the maximum rate is reached. The maximum and steady rates are higher. The total evolution of carbon dioxide is 30-40% greater than in the control which had been dried at 45°. Parallel experiments also showed a greater rate of esterification of the inorganic phosphate in the presence of dextrose.

Summary. Storage of yeast at 5° varies the fermentative activity of zymin made therefrom. The activity increased up to 14 days' storage but the preparation from yeast which has been stored 20 days was inactive. Drying the preparation in vacuum, instead of the usual temperature of 45° markedly increases the activity of the zymin. The increase in activity with storage of the yeast or by employing vacuum drying is correlated with increased rate of esterification of the inorganic phosphate of the preparation in the presence of dextrose.

8424 C

Effect of Aeration and Lack of CO₂ on Growth of Bacteria-Free Cultures of Protozoa.*

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It has been known for some time that CO₂ is necessary for the growth of bacteria, yeast, and molds on solid media^{1, 2} and recent

* Aided by a grant from the Rockefeller Foundation for work on the physiology of the normal cell.

¹ Valley, G., and Rettger, L. F., *J. Bact.*, 1927, **14**, 101.

² Valley, G., *Biol. Rev.*, 1928, **3**, 209.

investigations have shown that it is also necessary for bacteria when synthetic liquid media are used.^{3, 4} In liquid media containing hydrolyzed protein growth is not completely inhibited, but it may be delayed considerably by the absence of CO₂.^{4, 5} It has also been suggested that lack of sufficient CO₂ might be a cause of the lag period in growth of cultures of free-living protozoa and a cause of the failure of various investigators to obtain bacteria-free cultures of intestinal species.⁶ For these reasons information concerning the effect of lack of CO₂ on protozoa seems highly desirable.

In the present experiments bacteria-free cultures of *Glaucoma piriformis*† and *Chilomonas paramecium*‡ were grown in a heavily buffered medium of 0.5% hydrolyzed casein and 0.25% KH₂PO₄ at pH 6.0 under the following conditions: (a) unaerated, in flasks with cotton stoppers, (b) aerated, in Drechsel gas washing bottles with ordinary air which had been washed through 2 bottles of water and an indicator solution (brom thymol blue), (c) aerated, in Drechsel bottles with CO₂-free air prepared by passing air through 2 Milligan spiral gas washing bottles of strong KOH and then through 2 bottles of water and an indicator solution. These conditions are similar to those used by Walker⁵ for bacteria. The rate of bubbling was about 5 liters per hour per flask. The cultures were immersed in a stirred water bath, and the temperature was thereby maintained equal in all cultures and approximately that of the room (20°-25°C.). Each experiment was performed in duplicate and the results averaged. Counting methods used have been described previously.⁷

The experimental results are shown in Fig. 1. The ratio of the number of organisms present (x) at any time to the initial number (x_0) is plotted on a logarithmic scale with the value of $\log x/x_0$ marked on the left and the value of the ratio on the right ordinate; the abscissa is time in days. The data for *Glaucoma* represent a typical experiment. The aerated cultures grew more slowly during

³ Walker, H. H., *Science*, 1932, **76**, 602; *J. Bact.*, 1932, **14**, 169.

⁴ Gladstone, G. P., Fildes, P., and Richardson, G. M., *Brit. J. Exp. Path.*, 1935, **16**, 335.

⁵ Winslow, C.-E. A., Walker, H. H., and Sutermeister, M., *J. Bact.*, 1932, **24**, 185.

⁶ Jahn, T. L., *Cold Spring Harbor Symposia on Quant. Biol.*, 1934, **2**, 167.

† Strain isolated several years ago by Dr. Alford Hetherington of Stanford University.

‡ Strain isolated by present author and Dr. John B. Loefer of New York University in 1931.

⁷ Jahn, T. L., *Biol. Bull.*, 1929, **57**, 81; 1931, **61**, 387.

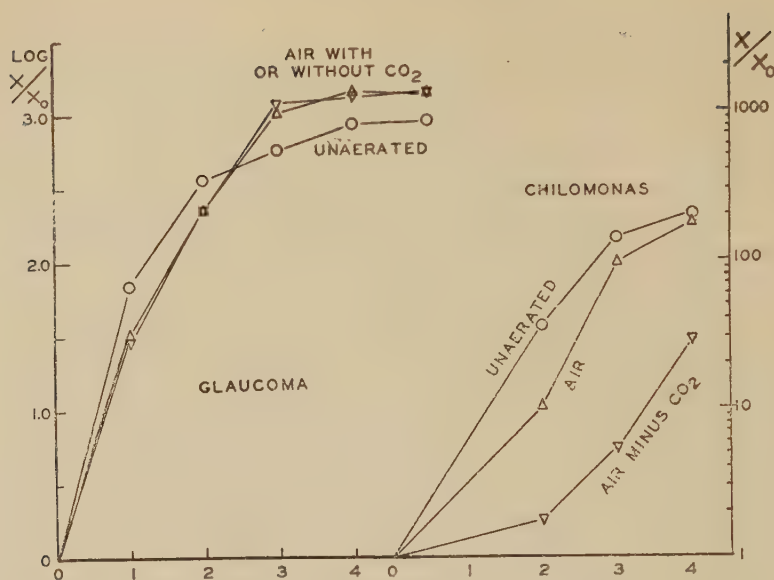


FIG. 1.

Graph showing the growth of cultures of *Glaucoma* and *Chilomonas* under different conditions. Ordinate: $\log x/x_0$, where x_0 is the initial number and x is the number present at any given time. Value of the log is given on the left and value of the ratio on the right. Abscissa: time in days.

the first day, but the final number in these cultures was about 60% higher than that in the aerated cultures. It was found that after the second day the organisms in unaerated cultures were about 50% larger than during the first 2 days and that the protoplasm was high vacuolated. Those in the aerated cultures showed no gross morphological changes during the course of the experiment. The higher total number and lack of morphological changes in the aerated cultures are probably due to the maintenance of a constant gaseous content. § There was no significant difference (*i. e.*, 15% or more) between the rate of growth in cultures aerated with ordinary air and those aerated with CO₂-free air. In a heavily buffered casein peptone medium, therefore, lack of CO₂ apparently had no effect on the growth of *Glaucoma*. Also, it is to be noted that no lag period was apparent, the rate of growth (slope of $\log x/x_0$ -time curve) being highest on the first day.

The data in Fig. 1 for *Chilomonas* represent the average values for 5 experiments, each of which was performed in duplicate. The unaerated cultures showed the highest initial growth rate and no

§ This does not occur in unaerated cultures of rapidly growing protozoa. (Jahn, T. L., *Arch. f. Protistenk.*, 1935, in press.)

apparent lag period. Those aerated with ordinary air showed a lower initial rate and a lag period. Those aerated with CO₂-free air showed the lowest initial growth rate and a more distinct lag period. No appreciable pH difference (greater than 0.1 unit) could be detected between cultures with or without CO₂. Lack of CO₂, therefore, causes a lower initial growth rate and a prolongation of the lag period in cultures of *Chilomonas*.

An explanation of why CO₂ should be necessary for growth of microorganisms has not been given in the literature. Gladstone, Fildes, and Richardson⁴ considered several possibilities but offered no theory to explain the phenomenon. It seems quite evident from the literature that the optimum concentration of CO₂ for bacteria varies widely for the different species, and on the basis of available evidence this also seems to be true for protozoa.|| We might expect, therefore, a wide variation in the effect of almost complete removal of CO₂ such as has been found with bacteria and in the present experiments. Since cell membranes are freely permeable to CO₂ it seems a natural assumption that the bicarbonate buffering system in the protoplasm of cells which are adapted to living in a medium of high CO₂ content should be greater in magnitude and, therefore, relatively more important for the maintenance of the intracellular pH than that of cells which ordinarily do not live in high CO₂ tension. If all of the CO₂ is removed from the medium, CO₂ will also be drawn from the protoplasm, thereby causing a shift in the bicarbonate buffer system and possibly an appreciable change in pH. If the bicarbonate system is of minor importance this change might be negligible, but if bicarbonate is the principal buffer involved the change would be of considerable importance. It seems, therefore, that this might be the key to an explanation of the necessity of CO₂. The effectiveness of the removal of CO₂ from the medium in removing CO₂ from the cell will depend, of course, on the rate of production of CO₂ by the organism which will vary with different media and with O₂ tension.⁴

On this theory one should expect CO₂ to be replaceable to some extent by other weak acids which are partly dissociated at the pH of the medium. The ionic fraction of such acids might be inhibited electrically from passing through the membrane, whereas phosphoric acid at the pH of the medium is completely in the form of ions, and

|| See Jacobs, M. H., (*J. Exp. Zool.*, 1912, **12**, 519) for differences in higher limits and present data for lower limits of CO₂ effect. *Chilomonas* is found in greatest numbers in very putrid cultures which undoubtedly have a high CO₂ content.

therefore, probably highly impermeable. Certain amino acids, however, are partially undissociated and probably permeable and therefore should decrease or prevent the deleterious effects of CO₂-free media. The necessary concentration of the substitute acid should vary with the CO₂ tension otherwise necessary for the species, and since the permeability of the cell to amino acids is probably considerably lower than to CO₂ complete substitution might not be possible in some cases. This fits the experimental evidence in that amino acids may partially replace CO₂ for growth of certain bacteria but that they seem ineffective for others⁴ (anaerobes or anaerobic conditions). In all aerobic conditions tried, however, CO₂ can be partially replaced by hydrolyzed protein.^{3, 4, 5} This might be due to a higher concentration of effective acids and/or to the higher production of CO₂ by the bacteria in these media. This theory seems to explain satisfactorily all the known data on the lack of CO₂ on microorganisms. Final proof, however, will involve measurements of the intracellular pH under various CO₂ tensions.

Pacific Coast Section

University of California Hospital, December 4, 1935.

8425 P

Hypochromic Anemia in Gastrectomized Dogs. Effect of Beef, Iron, Liver Extract on Hemoglobin.*

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From the Division of Medicine, University of California Medical School, San Francisco

We¹ have previously reported that patients with achlorhydria and hypochromic anemia fail to show increased hemoglobin formation upon the daily ingestion of a diet rich in food iron. However, with the feeding of a similar meal previously digested *in vitro* with hydrochloric acid and pepsin, satisfactory hemoglobin production did occur. In addition, a group of patients with a similar type of anemia responded to large doses of iron. It was concluded from these studies that chronic idiopathic hypochromic anemia was presumably due to a deficiency of iron wherein gastric dysfunction led to a failure in utilization of organic (dietary) iron.

Another group of experiments² completed in our laboratory show that in dogs gastrectomy greatly decreased the ability to form hemoglobin from beef protein. The results of such an experiment are recorded in Fig. 1. It is to be noted that the average daily output of hemoglobin was about 0.86 gm. while the dogs were on the standard bread ration, but was increased to 2.26 gm. when 250 gm. of beef were added to the diet. Following gastrectomy the hemoglobin was reduced to an average daily output of 0.4 gm. and 0.21 gm., on the standard bread diet and beef diet respectively.

The present study was undertaken to determine the effects of pre-

*Supported by a grant from the Christine Breon Fund for Medical Research.

† Research Fellow of the American College of Physicians.

¹ Mettier, S. R., Kellogg, F., and Rinehart, J. F., *Am. J. Med. Sci.*, 1933, **186**, 694.

² Kellogg, F., Mettier, S. R., and Purviance, K., unpublished.

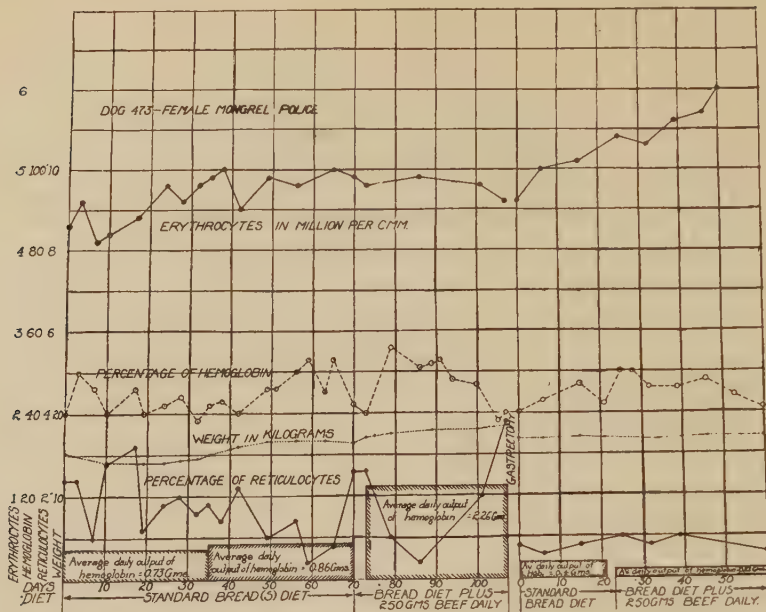


FIG. 1.

Effect of standard bread (S) and of beef on hemoglobin production before and after gastrectomy.

digested beef, of iron, and of liver extract on hemoglobin production in the same series of gastrectomized dogs.

The animals used in this study were maintained in a condition of basal production of hemoglobin by bleedings frequent enough to keep a hemoglobin level of from 6 to 9 gm. per 100 cc. of blood. The hemoglobin output over a given period of time was estimated by calculating the amount of hemoglobin removed at each bleeding, according to the method of Whipple. Total blood volume determinations were made by the method of Keith and Rowntree as modified by Hooper, Smith, Belt and Whipple.³

The standard bread ration of Whipple supplemented by salmon was used as the basal diet. The predigested meal consisted of from 250 to 750 gm. of beef digested *in vitro*, administered daily.

Tube feeding was resorted to because of the dogs' reluctance to eat voluntarily the predigested meal. Frequently a considerable amount of the meal was regurgitated. It is to be noted in Table I that hemoglobin formation was not augmented by this type of therapy. This we believe was due largely to the failure of the animals to retain the entire meal.

³ Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **51**, 205.

TABLE I.

Effect of Standard Bread (S), of Predigested Beef, of Iron and Ammonium Citrate and of Liver Extract on Hemoglobin Production in Gastrectomized Dogs.

Dog No.	No. of days	Hemoglobin Output gm. per day	Diet and Type of Therapy
473	20	0.20	Bread (S)
	20	0.24	750 gm. predigested beef
	20	1.73	Bread (S) + 1 gm. iron and ammonium citrate daily
	24	0.60	Liver extract, 5 cc. 3 times a week*
394	20	0.04	Bread (S)
	20	2.77	Bread (S) + 1 gm. of iron and ammonium citrate daily
	20	0.04	Bread (S) + 5 cc. liver extract daily
393	20	0.10	Bread (S)
	20	0.11	250 gm. of predigested beef
	20	1.85	Bread (S) + 1 gm. of iron and ammonium citrate daily
	20	0.19	Bread (S) + 5 cc. liver extract daily

*The authors wish to thank Eli Lilly and Company for their generous contribution of Liver Extract 343 (N.N.R.).

The daily administration of one gram of iron and ammonium citrate had a marked effect on hemoglobin regeneration. Prior to therapy the hemoglobin output in each case was less than 0.25 gm. daily, whereas after iron therapy it was 1.73, 2.77 and 1.85 gm. respectively. The results of this study of iron therapy compare favorably with those reported by Dragstedt⁴ and his associates.

The anemia, as shown in Table I, did not respond to liver extract specific for pernicious anemia.

8426 P

Curare-Actions of *Erythrina Americana*.

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From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

The great scarcity of active curare prompted an investigation of *Erythrina americana*, which has been reported by Altamirano,¹

⁴ Dragstedt, C. A., Bradley, J. D., and Mead, F. B., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 58.

¹ Altamirano, *cit.*, U. S. Dispensatory, 1918, 20th Ed., p. 1375; Altamirano, 1876, and Altamirano and Dominguez, *Gaceta Medica*, 1888, *cit.*, Arzac-Behnken, *loc. cit.*

Arzac-Behnken,² and Ramirez and Rivero³ to possess paralytic actions typical of curare. According to Altamirano, the paralytic and anticonvulsant actions are powerful. The active constituent appears to be an alkaloid, erythroidine, an emetic action being ascribed to erythroresin. Unfortunately, however, details of Altamirano's work are lacking, only an abstract being available to me. Arzac-Behnken claims to have confirmed the curare-like action in a few animals, but without proof. Poisoned animals do not recover from the paralysis, even when given artificial respiration, and pulmonary congestion and subacute nephritis are produced, according to Arzac-Behnken. Ramirez and Rivero report chemical analysis of the drug, and motor nerve paralysis in frogs by chronaxie determinations. This is about the extent of the pharmacologic literature on the drug. Therefore, a complete pharmacodynamic study was planned to assess the validity of the claims, and, if confirmed, the possibilities for the drug. The results obtained are sufficiently encouraging to justify this preliminary report.

The product* used was a liquid alcoholic extract of the seeds, containing 60% alcohol, so that 1 cc. represented 130 mg. of the crude drug. This was diluted with an equal volume of physiological salt solution (0.9% NaCl) before injection. A total of 76 animals of different species was used.

Fatal doses. In animals without artificial respiration, the average fatal doses in mg. of crude drug per kg., body weight are as follows: white mice, 600 (intraperitoneal); white rats, 675 (intraperitoneal and 100 intravenous); rabbits and pigeons, 5 (intravenous); pigeons, 50 (intramuscular); frogs, 500 (lymph sac; complete paralysis).

Sciatic nerve paralysis. This is complete after the following doses in mg. per kg., body weight for different time periods: frogs (lymph sac) and turtle (under plastron), 40 for 8 hours; mice, 600 for 45 minutes (intraperitoneal); rats, 600 for 20 minutes (intraperitoneal) and for 1 hour (intravenous); cats, 2.5 to 5 for 1 to 2 hours (intravenous); rabbits and pigeons, 50 for 2 hours (intra-

² Arzac-Behnken, "Estudio Comparativo de la Erythrina Americana (Colorin Comun) con el Curare," Tesis, Universidad Nacional Autonoma, Mexico, D. F., 1934, p. 47.

³ Ramirez and Rivero, "Contribucion al Estudio de la Accion Farmacodinamica de la Erythrina Americana," 1935 (Lab. Fac. de Med. Univ. Nal. de Mexico), personal communication from Mr. G. G. Colin.

* The product was generously supplied by Mr. G. G. Colin of the Laboratorio Quimico Central, S. A., Mexico, D. F.

venous); dog, 20 for 6 hours (intravenous). (Artificial respiration in higher animals.)

Skeletal muscle-nerve preparation. The classical Claude Bernard experiment gave completely positive results as follows: concentrations of 1:10,000, ineffective; 1:5000, effective, causing complete nerve block in 30 minutes and the muscle substance remaining unpoisoned; 1:2500, no more effective than 1:5000.

Phrenic nerve paralysis. In pigeons, rabbits, and cats, a regular, though shallow, thoracic type of respiration continued almost invariably after sciatic nerve paralysis was complete with doses of 5 mg. per kg. Using the procedure of Thomas and Franke,⁴ on cats and rabbits, in which contractions of innervated segments of the diaphragm are recorded simultaneously, one segment being *in situ*, and one, *in vitro* without blood circulation, it was conclusively demonstrated that the seat of phrenic paralysis is peripheral. Respiratory paralysis occurs as the result of asphyxia, but, if asphyxia is prevented by artificial respiration, recovery of natural respiration is complete after 10 to 15 times the average dose for sciatic paralysis, contrary to Arzac-Behnken's claim.

Circulation. In cats, rabbits, rats, and dogs, intravenous injection of erythrina causes a typical fall of blood pressure with decrease in volume of viscera and increase in volume of legs as the result of relaxation of skeletal muscles, as with curare. Recovery is gradual but prompter than with curare. In pigeons, there is a sharp but fleeting rise of blood pressure, like that of epinephrine, as the result of a brief peripheral vasoconstriction (legs): difference from curare, which causes a fall of pressure.

Anticonvulsant action. Using surely fatal doses of strychnine in rats, rabbits, pigeons and a dog (4 times the fatal dose), the typical convulsions were prevented by prior intramuscular injections of erythrina, or abolished immediately by intravenous injections. Using artificial respiration continuously, or as needed, complete recovery and great amelioration of seizures may be obtained, depending on the dose of strychnine, but without the aid of artificial respiration recovery from the highest doses is not yet assured.

Conclusions. Claims that *Erythrina americana* possesses a typical curare action are confirmed, proven, and extended for several species of animals. General pharmacodynamic analysis indicates tentatively a promising usefulness for this drug as a comparatively easily available and practical substitute for curare and as a possible anticonvulsant in poisoning and in excitatory states.

⁴ Thomas and Franke, *J. Pharm. Exp. Therap.*, 1928, **34**, 111.

Age and the Qualitative Ovarian Response of the Immature Rat
to Mare Gonadotropic Hormone.

F. J. SAUNDERS AND H. H. COLE.

From the College of Agriculture, University of California, Davis.

It was shown¹ that 21- and 25-day-old rats responded to mare gonadotropic hormone with follicular growth, ovulation, and the formation of corpora lutea. Later it was shown² that in female rats 10 days old at the time of the injection the response consisted in a marked development of interstitial tissue. This divergence in results led us to study further the nature of the response dependent upon age.

Rats were divided into groups with age and size of the dose as the variables. Single doses of 2, 16, and 500 R. U. were given to rats 15, 18, 19, 21, and 25 days old at the time of injection with necropsy 120 hours after the injection. The ovaries and uteri were weighed and the ovaries of three rats in each group were sectioned serially. The response of the 15-day-old rats is comparable to that of 10-day-old rats and thus does not need further consideration.

A pure follicle stimulating response was obtained at all dosage levels with rats injected on the 18th day. Not a single corpus luteum was found in 26 rats injected at this age. Macroscopically enlarged pale translucent follicles were visible. Microscopic study shows that ovulation has not occurred and there is no evidence of the conversion of the granulosa into lutein tissue. There is, however, a marked development of interstitial tissue in rats receiving the larger amounts, which is in many ways similar to that found in the ovary of the rabbit in heat. In those receiving 2 R. U. the only detectable change is the growth of a few follicles.

Ovulation and the formation of corpora occurred in 3 of 18 rats injected on the 19th day of age, and the results in 21- and 25-day-old rats were comparable to those previously obtained.

The question arises as to why this difference in response dependent upon age is obtained. One might assume that a luteinizing substance is secreted by the pituitary in the 25- but not in the 18-day-old rat, thereby accounting for luteinization in the older rat. Casida³ obtained some results in favor of this view. He injected extracts of

¹ Cole, H. H., Guilbert, H. R., and Goss, H., *Am. J. Physiol.*, 1932, **102**, 227.

² Cole, H. H., *Am. J. Anat.*, in press.

³ Casida, L. E., *Endocrinology*, 1934, **18**, 714.

gelding pituitaries into 2 strains of rats. In one a pure follicle stimulating response was noted whereas in the other ovulation occurred with the consequent formation of corpora lutea. When a small amount of pituitary luteinizer was added to the gelding pituitary extract and injected into the first mentioned strain ovulation occurred. Against such a view, however, is the fact that Smith⁴ was able to produce both follicular growth and luteinization in hypophysectomized rats with mare gonadotropic hormone. This, it seems, should be conclusive proof that luteinization following mare gonadotropic hormone treatment is not dependent upon a factor secreted by the pituitary of the recipient. If this is true, then the difference in response must be explained either on the basis of the state of the ovary at the moment of injection or by assuming that a second factor is secreted elsewhere in the body.

The results given in this paper point out clearly the necessity of knowing the stage of sexual development of rats used in studying the qualitative response to gonadotropic substances. As different strains of rats mature at different ages it is obvious that different responses might be obtained in 2 strains of rats even though their ages were the same. This may to some extent explain the divergent results obtained with pituitary extracts in different laboratories.

8428 P

Means of Augmenting the Ovarian Response to Gonadotropic Substances.

F. J. SAUNDERS AND H. H. COLE.

From the College of Agriculture, University of California, Davis.

We presented evidence¹ that the serum of the pregnant mare contains 2 substances, one which produces sexual maturity and fully formed corpora lutea in the immature rat and another which is ineffective by itself but is capable of augmenting the response to pituitary synergist prepared after the method of Evans, *et al.*² We obtained augmentation of this synergist by the use of a fraction prepared from mare serum after the method described by Fevold

⁴ Smith, P. E., *J. Am. Med. Assn.*, 1935, **104**, 553.

¹ Saunders, F. J., and Cole, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1476.

² Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

*et al.*³ for the preparation of luteinizer from the pituitary. Cole and Hart⁴ obtained augmentation of this synergist by serum from non-pregnant mares. Evans⁵ obtained augmentation of his synergist by combining it with sera from pregnant and non-pregnant individuals of various species. From evidence now at hand it appears that the augmentation produced by these sera or their extracts is due not to a specific gonadotropic substance but rather to their protein content. Similar augmentation can be produced by a wide variety of inert materials.

Augmentation by inert materials was first reported by Fevold³ in the preparation of his follicle stimulator when he stated that precipitation of the active material on tannic acid and injection of the tannate in colloidal solution accentuates the ovarian response. Maxwell⁶ working with the hypophyseal gonadotropic substance, was able to obtain increased response by the addition of ZnSO_4 to the crude pituitary extract. We have been able to confirm Maxwell's work, obtaining an average increase of about 100% in ovarian weight by the addition of ZnSO_4 to the crude pituitary extract.* Furthermore using pituitary synergist (Evans' method) we have obtained a 3-fold increase in ovarian weight by the addition of ZnSO_4 and also significant increases by the addition of casein or egg albumin.

In making the solutions for these experiments the materials were weighed out and dissolved in an amount of saline such that 6 ml. contained the total dose. The mixtures were allowed to stand for several hours before the first injection. They were then administered in 3 daily subcutaneous injections starting when the rats were 25 days old with necropsy 5 days after the first injection.

From Table I it will be noticed that while significant augmentation is obtained with egg albumin and casein, that obtained with ZnSO_4 is much greater. Increasing the amount of egg albumin from 10 to 60 mg. produced no significant change in response. The addition

³ Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, **104**, 710.

⁴ Cole, H. H., and Hart, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 370.

⁵ Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

⁶ Maxwell, L. C., *Am. J. Physiol.*, 1934, **110**, 458.

* The crude pituitary extracts were prepared by extraction of the dried gland with 6% butyl alcohol, concentration of the extract under reduced pressure and precipitation of the residue with excess acetone after the method of Meyer and Fevold.⁷

⁷ Meyer, A. E., and Fevold, H. L., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 570.

TABLE I.
Effects of Combining Pituitary Synergist with Various Inert Materials.

Material	No. of animals	Aver. ovarian wt., mg.	Significance of increase over synergist alone	
			t	P
Synergist alone	12	34.0 ± 5.8		
" + ZnSO ₄ · 7H ₂ O (9 mg.)	6	104.7 ± 40.1	5.2	0.01 > P
" + casein (10 mg.)	6	44.7 ± 6.8	3.3	0.01 > P
" + egg albumin (10-60 mg.)	12	55.4 ± 7.6	9.2	0.01 > P
" + casein (10 mg.)				
+ ZnSO ₄ · 7H ₂ O (9 mg.)	6	106.5 ± 33.6	5.0	0.01 > P

The same dose of synergist (2 mg.) was used in all cases. Values of P less than 0.05 may be considered significant.

of casein to the synergist-zinc sulfate mixture produced no further augmentation. Similarly addition of ZnSO₄ to untreated mare serum, in doses containing 11 mg. of protein, was without effect. The questions arise, how are these substances acting and are they all acting in the same manner? Fevold explained the augmentation of the follicle stimulator response by tannic acid as being due to the action of the latter in delaying absorption. Maxwell used the same explanation for the effect of ZnSO₄ on the crude pituitary extracts and presented quite conclusive evidence in favor of this view. The same explanation might hold equally well as regards the effect of protein. Cole, Guilbert and Goss⁸ found that mare serum, with its relatively high content of protein, was just as effective when administered in a single dose to 25-day-old rats as when the dose was divided into 8 injections over a period of 4 days. As to the second question, whether or not all these substances are acting in the same manner, we have little conclusive evidence except that mare serum, which contains considerable protein, is not activated by ZnSO₄, casein did not increase the response when added to the synergist-zinc sulfate mixture, and no significant difference in qualitative response is evident when different agents are used as augmenting principles. In all of these cases in which we obtained an augmented response, the ovaries were heavily luteinized. Therefore it seems logical to assume that all these substances are acting in the same manner, possibly by delaying absorption of the active principle. The relationship between the augmenting principles and the pituitary luteinizers is not clear. In our hands, both the augmenting substances herein discussed and the pituitary luteinizer augment the response to certain pituitary fractions and both, in conjunction with these fractions, produce heavily luteinized ovaries. The point is far from being

⁸ Cole, H. H., Guilbert, H. R., and Goss, H., *Am. J. Physiol.*, 1932, **102**, 227.

settled, however, and it should be stated that work in other laboratories points toward a more specific effect of the pituitary luteinizer.

8429 P

Quantitative Interpretation of Biological X-Ray Effects.*

FRED M. UBER. (Introduced by S. C. Brooks.)

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In dark-field photomicrographs¹ of microincinerated pollen mother cells of the lily, the chromosome ash is most conspicuous against the black background which results from the paucity of ash in the karyolymph or nucleoplasm. Animal cells yield a similar picture (Scott,² Kruszynski³). This marked concentration in the chromosomes of elements of comparatively high atomic weight, as potassium, calcium and iron probably and phosphorus certainly, may be related quantitatively to X-ray phenomena in at least 3 ways.

First, rough calculations based on microincineration evidence and on the composition of nucleic acid indicate that the mass absorption coefficient of chromatin is more than double that of air and, hence, that of nucleoplasm. This relatively greater absorption of radiant energy by the chromosomes may account in large measure for the greater sensitivity of the nucleus compared to the cytoplasm, an experimental fact confirmed by a number of investigators.

Second, the amount of chromatin in a "sensitive volume," a concept associated with the quantum hit theory of radiation injury, would be but a fraction of that computed for it on the ordinary assumptions, *i. e.*, those which underlie the definition of the roentgen as a dosage unit. Were the validity of the theory established and relevant measurements precise enough, this size factor might prove important in assigning the "sensitive volume" to a definite cytological entity.

Third, the localization of nuclear ash in the chromosomes should manifest itself in a biologically detectable wave-length effect at

* Part of investigation supported by grant of the Rockefeller Foundation to Prof. T. H. Goodspeed.

¹ Uber, F. M., and Goodspeed, T. H., *Bot. Gaz.*, 1935, **97**, No. 2, in press.

² Scott, G. H., *Bull. d'Hist.*, 1930, **7**, 251.

³ Kruszynski, J., *Bull. internat. Acad. Polonaise, Cl. Sci. Math. et Nat.*, 1934, **2**, 105.

the absorption discontinuity of any comparatively heavy element present in relative abundance. The fact that the wave-lengths for the elements concerned lie in the range 3-6 Angström units probably explains why no such effect has been discovered to date. Conversely, if a sufficiently sensitive criterion of biological effect could be found, perhaps some genetic change, it would serve as a test for the presence of specific heavy elements in the hereditary material.

8430 P

A Quantitative Study of the Cochlear Response.*

L. J. BLACK AND W. P. COVELL. (Introduced by K. F. Meyer.)

From the Laboratories of the Electrical Engineering Department and the George Williams Hooper Foundation for Medical Research, University of California.

Quantitative measurements of the cochlear response in animals have been part of a program concerned with investigations on hearing problems during the past year and one-half in these laboratories. The methods and apparatus used for the detection of the responses was similar to that described by Garceau and Davis.¹ An accurate system for the measurement and generation of pure stimulation tones was essential. An audio-frequency oscillator generated electrical voltages for the production of these tones. The voltages were amplified, measured, attenuated to the desired level and finally transformed into sound by a Western Electric 555 speaker unit. The harmonic content of the sound wave as measured by means of a Brush crystal microphone, amplifier and General Radio Distortion Factor meter was never more than one per cent for tones below 4000 cycles per second. The total power of the harmonics was therefore 40 decibels below that of the fundamental in this range. A fabric reinforced rubber tube 6 feet in length conveyed the sound to the animal's ear.

The stimulus was calibrated in terms of microwatts of acoustical energy. This was accomplished by measuring the power level to the speaker necessary to produce threshold stimuli for each ear of 6 humans. The mean value of these thresholds was considered

* Aided by a grant from the John C. and Edward Coleman Memorial Fund, University of California.

¹ Garceau, E. L., and Davis, H., *Am. J. Physiol.*, 1934, **107**, 305.

identical to the "minimum audible pressure" threshold values as determined by Sivian and White.²

The electrical voltages generated in the cochlea of the animal's ear were amplified and recorded with an electrode placed on the round window and an indifferent electrode buried in the muscles of the neck. The low level amplifier consisted of 4 stages which were resistance capacity coupled. A set of electrical filters made it possible to measure any frequency component to the exclusion of all others. These filters were necessary in the "masking" studies and their use also extends the limit of the smallest measurable input voltage.

For oscillographic purposes any one of 3 oscillographs were available, the choice depended upon the particular test.

The response for each frequency reaches a definite maximum value (Davis³). Over a considerable range of intensities the response in microvolts is practically proportional to the sound pressure. This linear relation holds from very low values of stimulation, presumably threshold values, to intensities within approximately 20 decibels of the intensity that gives the maximum cochlear response. Over this range of intensities the wave form of the stimulus is faithfully reproduced in the response and the harmonic content is less than 5%. The sensitivity of the response at 1000 cycles is 65 microvolts per dyne per sq. cm. The departure from this linear relation at high intensities of stimulation resembles the departure noted when any mechanical device is overloaded.

The rate of increase of response with increase of stimulus is greater for tones below one thousand cycles per second than for those above. This characteristic is probably associated with the greater change of loudness for a given change of intensity for low tones (Fletcher⁴).

The curve obtained by plotting the stimulus necessary to produce the maximum response as a function of the tone frequency resembles approximately the threshold of feeling curve (Wegel⁵). The maximum response at 1000 cycles is reached for a stimulus 18 decibels below the human threshold of feeling. This was checked by having several observers place the same tube used in testing the

² Sivian, L. J., and White, S. D., *J. Acoust. Soc. Am.*, 1933, **4**, 288.

³ Davis, H., *A handbook of general experimental psychology*. C. Murchison, Clark Univ. Press, Worcester, Mass. 1934, pp. 962-986.

⁴ Fletcher, H., *Speech and Hearing*. D. Van Nostrand Co., Inc., New York, 1929. p. 226.

⁵ Wegel, R. L., *Ann. otol., rhinol. and laryngol.*, 1932, **41**, 740.

cats in their own external canals and increasing the stimulus until discomfort was experienced. The results suggest that the threshold of feeling occurs at a lower stimulus for a cat than for a human.

Contours of equal output, obtained by plotting the stimulus necessary to give various values of response as a function of the tone frequency resemble the familiar human equal loudness curves of Kingsbury (Fletcher⁴). Although the one microvolt (effective value) curve is about 20 decibels above the human threshold of audibility, the response can be detected for intensities of stimulation very close to the human threshold of hearing.

The presence of any tone decreases the cochlear response to any other tone. The percentage decrease of the response to any tone increases with the intensity of the interfering tone. It was thought that perhaps a detailed study of the relative decrease caused by various interfering frequencies would reveal information concerning subjective masking. The results, however, revealed that the percentage decrease of the response to any tone was the same for a high frequency interfering tone as for a low frequency interfering tone. There was no correspondence to subjective masking of high frequencies by low frequencies. The maximum total response to 2 tones (250 to 3000 cycles) does not proportionally exceed the maximum response due to either tone alone.

Missouri and Southern Sections

Municipal Auditorium, St. Louis, November 19, 1935.

8431 P

Recovery of Mammalian Nerve Fibres in Vivo.

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An isolated nerve fibre, as it recovers from the condition of absolute refractoriness which follows activity, is known under suitable conditions to pass through 3 successive phases, relative refractoriness, supernormality, and subnormality.^{1, 2, 3} We have attempted to determine the course of recovery of nerve fibres in as nearly as possible physiological conditions, *i. e.*, those conditions existing in the body of a healthy, intact animal. Etherized or decorticated rabbits were used, and induction shocks were applied either to the oculomotor nerve *in situ*,⁴ or to the sciatic nerve at the level of the trochanter major of the femur. The greatest care was taken to prevent hemorrhage, tissue injury or temperature change. Responses were recorded by means of a cathode ray oscillograph from the internal rectus muscle, the nerve to the inferior oblique muscle, the tibialis or gastrocnemius muscle or the deep peroneal nerve, according to the nerve stimulated. Thirty experiments were performed.

The recovery curves obtained often included the three phases of relative refractoriness, supernormality and subnormality, but there was considerable variation from nerve to nerve, and in the same nerve from time to time. Apparently the nerve fibers are extremely sensitive to metabolic changes taking place in the body, so that the recovery curve varies continuously. The relatively refractory period usually ended 2-5 msec. after the conditioning shock, and

¹ Adrian, E. D., and Lucas, K., *J. Physiol.*, 1912, **44**, 68.

² Graham, H. T., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 193; *Am. J. Physiol.*, 1935, **111**, 452.

³ Gasser, H. S., *Am. J. Physiol.*, 1935, **111**, 35.

⁴ Lorente de Nó, R., *Am. J. Physiol.*, 1935, **112**, 595.

was succeeded by a supernormal period lasting until 10-20 msec. after the shock, with a peak of excitability at 4-7 msec. At the peak, the maximum change in excitability (as measured by the change in strength of stimulus required to bring the response to normal height) was 5-10%. There was no striking difference between the oculomotor and the sciatic nerves in regard to the refractory and supernormal periods, but the subnormal period was much more prominent in the latter. This period did not outlast 35 msec. with the oculomotor nerves, and was sometimes apparently absent altogether, while with the sciatic nerves it usually lasted 50-100 msec. (minimum excitability 3-4% below normal, occurring at 20-30 msec.) and was present all or part of the time in every experiment.

When the preparation was made rapidly so that the recovery curve could be obtained within an hour after fastening the rabbit to the board, and when the nerve was stimulated as few times as possible before observing the recovery at the shock intervals where supernormality ordinarily occurs, instead of supernormality prolonged refractoriness was found. This refractoriness then disappeared, usually very promptly, and supernormality or at least a peak of excitability developed at these intervals. The supernormal phase is therefore not present normally. On the other hand, it is also not present in moribund rabbits. As to how it is produced, our experiments are not conclusive. Since it has been found in freshly prepared nerves of rabbits of which other nerves had been under experimentation for some time, it can hardly be attributed to a local change in the nerve being stimulated. It was sometimes decreased by forced hyperventilation of the lungs; and following (though not during) asphyxia by rebreathing, it may increase very markedly. Ether does not seem to have a specific effect.

Of special interest is the fact that while the excitability of the motor fibres to induction shocks was supernormal at certain intervals, the excitability of the motoneurones to synaptic stimuli was subnormal at these same intervals; the recovery of excitability to synaptic stimuli always was as previously described.⁴ One might be tempted to explain this difference by the assumption that, although kept under identical conditions, the axon but not the soma of the motoneurones had been modified so as to manifest supernormality; however, when the excitability of the soma of the motoneurones was tested by induction shocks applied directly to the oculomotor nucleus,⁵ the recovery of normal excitability was as prompt

⁵ Lorente de N6, R., *J. Cell. Comp. Physiol.*, 1935, 7, 47.

as in the nerve fibre. Evidently at intervals at which the soma of the motoneurons has subnormal excitability for synaptic stimuli, it has normal and even possibly supernormal excitability for induction shocks.

8432 C

Use of Liver Extract Intramuscularly in the Course of Acute Amebiasis in Dogs.*

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Previous communications from this laboratory^{1, 2, 3} have demonstrated the amebostatic and possibly amebacidal properties of whole raw liver and of powdered liver extract administered orally to dogs which had been experimentally infected with human strains of *Endamoeba histolytica*. The present investigation deals with dogs, similarly infected and suffering from acute amebic enteritis, treated with liver extract intramuscularly. In 6 such animals a commercial product, generously furnished us by Parke, Davis and Co., was used; in a parallel series of 8 dogs we prepared our own sterile fresh liver extract. In both series 1 cc. of the extract represented 5 gm. of fresh pig's liver. Alternately 2 cc. of the extract was injected respectively into the right and left gluteus muscles. The results of the investigation, together with the clinical history of the animals and brief autopsy reports, are presented in the following protocols. In all cases the same human strain of the organism, previously passed through several dogs, was utilized. We are indebted to Col. Chas. F. Craig for certain complement fixation tests on these dogs.

Dog 211

Nov. 2, 1934 Inoculated intracecally with trophozoites (T) of *E. histolytica* obtained from dysenteric exudate of dog 210; animal healthy, about one year old.
 " 5 *E. histolytica* T+ first recovered by intracecal aspiration; stool diarrheic.

*Aided by a grant from the David Trautman Schwartz Research Fund.

¹ Kagy, E. S., and Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 252.

² Faust, E. C., and Kagy, E. S., *Am. J. Trop. Med.*, 1934, **14**, 235.

³ Faust, E. C., Scott, L. C., and Swartzwelder, J. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 540.

- " 7-10 Stools semi-dysenteric; *E. histolytica* T+++; r.b.c., (Nov. 8) 4,765,000, (Nov. 10) 4,480,000; Hb % (Tallqvist), 70.
- " 12 Infection acute; marked tenesmus; stools dysenteric; animal losing weight; r.b.c., 4,325,000; given *liver extract* (2 cc.) intramuscularly.
- " 14 *Liver extract* (2 cc.); dehydration and emaciation marked; r.b.c., 4,515,000; Hb %, 55.
- " 16 *Liver extract* (2 cc.); infection fulminating; animal eats well but passes copious dysenteric stools; r.b.c., 3,310,000; Hb %, 50.
- " 18 *Liver extract* (2 cc.); infection still fulminating; *E. histolytica* T+++.
- " 19-20 Condition becoming grave; *E. histolytica* T+++; r.b.c., (Nov. 20), 1,780,000; Hb %, 45.
- " 21 Animal sacrificed; extremely emaciated and dehydrated; organs essentially normal except large bowel, which was riddled with many deep craterous amebic lesions, containing myriads of active *E. histolytica*.
- Dog 212
Nov. 3, 1934 Inoculated intracecally with trophozoites (T) of *E. histolytica*, obtained from dysenteric exudate of dog 210; animal about one year old, in excellent condition.
- " 6 First found positive (*E. histolytica* trophozoites in flecks of bloody mucus in stool).
- " 9 Stools dysenteric; *E. histolytica* T+++; tenesmus; animal losing weight; r.b.c., 5,905,000; Hb % (Tallqvist), 60.
- " 10 Condition the same; r.b.c., 6,125,000.
- " 12 Marked tenesmus; infection acute; *liver extract* (2 cc.) intramuscularly.
- " 13 Condition the same, r.b.c., 6,525,000.
- " 14 *Liver extract* (2 cc.); losing weight; marked tenesmus and slight anorexia.
- " 15 Animal becoming dehydrated; stool dysenteric, with many *E. histolytica* T; r.b.c., 6,930,000.
- " 16 *Liver extract* (2 cc.); condition acute.
- " 17 Animal becoming emaciated; marked tenesmus; stool bloody, diarrheic; *E. histolytica* T+++; r.b.c., 6,690,000.
- " 18 *Liver extract* (2 cc.); rapidly losing weight; stool dysenteric.
- " 19 Condition unchanged; r.b.c., 6,625,000.
- " 21 *Liver extract* (2 cc.); condition unchanged.
- " 22 r.b.c., 6,340,000; Hb %, 80.
- " 24 *Liver extract* (2 cc.); condition unchanged.
- " 25 Animal weak, inactive; condition acute; r.b.c., 6,025,000.
- " 26 *Liver extract* (2 cc.); stool dysenteric; *E. histolytica* T+++; r.b.c., 6,130,000.
- " 30 Infection continues to fulminate; r.b.c., 5,850,000.
- Dec. 3 Animal gravely ill; sacrificed; autopsy showed extreme emaciation; food undigested; all organs negative except large bowel, which was riddled with typical amebic lesions, containing myriads of active *E. histolytica*.
- Dog 213
Nov. 5, 1934 Inoculated similarly from dog 210; animal fat and active, about one year old.
- " 8 *E. histolytica* trophozoites (T) first found in flecks of blood in stool; r.b.c., 4,935,000; Hb % (Tallqvist), 60.
- " 9 Stool semi-formed to diarrheic, with flecks of blood and mucus; *E. histolytica* T+; slight tenesmus.
- " 10 Stool semi-dysenteric; *E. histolytica* T+++; r.b.c., 4,730,000.
- " 12 *Liver extract* (2 cc.) intramuscularly; r.b.c., 4,985,000.
- " 13 Infection acute; r.b.c., 5,165,000.
- " 14 *Liver extract* (2 cc.); condition unchanged; tenesmus; animal losing weight.

- " 15 r.b.c., 5,340,000.
 " 16 *Liver extract* (2 cc.); tenesmus and marked anorexia.
 " 17 r.b.c., 4,750,000.
 " 18 Condition fulminating; colon and rectum very hemorrhagic.
 " 19 *Liver extract* (2 cc.); r.b.c. 4,405,000; marked loss of weight.
 " 20 Animal refuses food; weak and inactive; r.b.c., 4,310,000.
 " 21 *Liver extract* (2 cc.); condition unchanged; complement fixation ++++.
 " 23 *Liver extract* (2 cc.); lower bowel profusely hemorrhagic; *E. histolytica* T++++.
 " 25 Animal rapidly growing worse, with blood freely flowing from rectum; r.b.c., 4,115,000; Hb %, 50.
 " 26 *Liver extract* (2 cc.); animal in grave condition.
 " 27 Animal in extremis, sacrificed; autopsy showed some emaciation; organs essentially negative, except large bowel, which showed numerous deep amebic ulcers in cecum, lower colon and rectum, but especially in appendix, which was almost perforated.
- Dog 220
 Nov. 24, 1934 Inoculated intracecally with *E. histolytica* trophozoites (T) from dog 213; dog active, healthy, about one year old.
 " 28 First positive by intracecal examination; *E. histolytica* T+ in bloody mucus.
 " 30 Bloody mucus in stool with trophozoites ++.
 Dec. 2 Marked tenesmus; animal eating well but losing weight.
 " 4 Condition becoming acute.
 " 5-8 No change in animal's condition.
 " 10 Animal becoming emaciated and dehydrated; tenesmus; *E. histolytica* T+++ in stool consisting mostly of dysenteric discharges.
 " 11-16 No change in animal's condition.
 " 18 *E. histolytica* T++ in bloody exudate; r.b.c., 4,265,000; Hb % (Tallqvist), 80.
 " 20 *E. histolytica* T+++; *liver extract* (2 cc.) intramuscularly; r.b.c., 4,115,000.
 " 23 r.b.c., 4,595,000; Hb %, 70.
 " 24 Infection acute; *liver extract* (2 cc.); r.b.c., 4,050,000; Hb %, 70.
 " 26 *Liver extract* (2 cc.).
 " 28 r.b.c., 4,445,000; Hb %, 60.
 " 29 *Liver extract* (2 cc.); r.b.c., 4,685,000; Hb %, 70.
 " 31 *Liver extract* (2 cc.); animal losing weight; tenesmus and anorexia; dysenteric stools numerous; r.b.c., 4,620,000; Hb %, 70.
 Jan. 2, 1935 Condition of animal acute but not fulminating; *liver extract* (2 cc.).
 " 3 Condition fulminating; r.b.c., 4,520,000; Hb %, 70.
 " 5 No change in condition.
 " 7 Animal sacrificed; complement fixation ++; at autopsy no essential pathology of organs except in large bowel in which appendix, mid-colon and rectum contained many relatively shallow amebic lesions, filled with active trophozoites.
- Dog 222
 Nov. 27, 1934 Inoculated intracecally with trophozoites (T) of *E. histolytica* obtained from dog 217; animal healthy, about 1 year old.
 " 30 First positive by intracecal tube; *E. histolytica* T+ in flecks of bloody mucus.
 Dec. 1-4 Infection becoming acute; animal fed 100 gm. chopped raw liver to control infection.
 " 5 Animal refuses regular food; given 200 gm. raw liver.
 " 7-9 Animal noticeably improved on raw liver by mouth.
 " 10-11 Animal given tinned salmon to exacerbate infection.
 " 12-19 Animal on regular diet; infection again acute; r.b.c., 6,645,000; Hb %, 80.

" 20	<i>Liver extract</i> (2 cc.) intramuscularly; condition still acute; r.b.c., 6,700,000; Hb %, 75.
" 22	<i>Liver extract</i> (2 cc.)
" 23	Many dysenteric stools a day; r.b.c., 6,745,000; Hb %, 75.
" 24	<i>Liver extract</i> (2 cc.)
" 26	<i>Liver extract</i> (2 cc.); animal losing weight; marked tenesmus; many dysenteric stools daily.
" 28	Condition unchanged; r.b.c., 6,350,000.
" 29	<i>Liver extract</i> (2 cc.); animal weak and inactive.
" 31	<i>Liver extract</i> (2 cc.); infection fulminating.
Jan. 2, 1935	<i>Liver extract</i> (2 cc.); r.b.c., 6,320,000; Hb %, 80; animal acutely ill.
" 3	r.b.c., 6,225,000; Hb %, 80.
" 5	r.b.c., 6,310,000; Hb %, 70.
" 7-9	Animal critically ill.
" 11	Animal sacrificed; complement fixation +++++; extremely emaciated; no pathology except in large bowel, which showed amebic invasion of entire length; lesions only moderately deep; myriads of active amebæ.
Dog 223	
Nov. 28, 1934	Inoculated intracecally with trophozoites of <i>E. histolytica</i> (T) from dog 217; animal healthy, about one year old.
Dec. 1	First positive by intracecal aspiration; <i>E. histolytica</i> T++ in flecks of blood and mucus.
" 3	Infection becoming acute.
" 4-5	Stool dysenteric; marked tenesmus; dog refusing regular food; becoming dehydrated and inactive.
" 6-11	Animal acutely ill with amebic dysentery.
" 12	Placed on diet of raw liver (100 gm. daily) by mouth to control infection.
" 17	Animal returned to regular diet after marked improvement in condition.
" 18	Infection exacerbating; marked tenesmus; some loss in weight; r.b.c., 4,700,000; Hb %, 65.
" 19	Condition subacute; r.b.c., 4,625,000; Hb %, 60.
" 20	Condition more acute; stools more dysenteric; <i>liver extract</i> (2 cc.) intramuscularly.
" 21	Condition unchanged; r.b.c., 4,730,000; Hb %, 60.
" 22	Dysentery still severe; <i>liver extract</i> (2 cc.).
" 24	Animal eating well but losing weight; stools dysenteric with many active amebæ; r.b.c., 4,600,000; Hb %, 50; <i>liver extract</i> (2 cc.).
" 26	Many dysenteric stools daily; dog losing weight and becoming inactive again; r.b.c., 4,715,000; Hb %, 60; <i>liver extract</i> (2 cc.).
" 28	Condition unchanged; r.b.c., 4,465,000; Hb %, 60.
" 29	Dysenteric condition fulminating; r.b.c., 4,235,000; Hb %, 60; <i>liver extract</i> (2 cc.).
" 31	Condition acute; marked tenesmus; r.b.c., 4,110,000; Hb %, 60; <i>liver extract</i> (2 cc.).
Jan. 2, 1935	Loss in weight appreciable; animal weak and inactive; r.b.c., 4,310,000.
" 5	No change in condition; r.b.c., 4,180,000; Hb %, 60.
" 11	Animal sacrificed; complement fixation +++++; some emaciation and dehydration; autopsy showed no essential lesions except in large bowel, which contained many moderately deep amebic lesions with numerous active <i>E. histolytica</i> .

Similarly a series of 8 dogs (224, 226, 227, 228, 229, 231, 233, 235) was inoculated with the same strain of *E. histolytica* by the intracecal method. All became positive; when the infection became

acute they were treated intramuscularly from time to time (at 2 to 3 day intervals) with 3 cc. freshly prepared sterile raw liver extract (expressed from chopped liver, passed through a Seitz filter and preserved aseptically, all at room temperature). While these animals showed some individual variations in their resistance to the amebic infection and while the quantity and quality of the erythrocytes was maintained at a significantly high level by the liver extract, there was no apparent control of the infection.

In all members of these 2 series, with the exception of dog 211, to which probably an inadequate amount of liver extract was administered, there was a remarkably high erythrocyte count and fair hemoglobin percentage in spite of the constant loss of blood due to profuse hemorrhage from the large bowel. In practically every animal the erythrocyte count increased after liver extract therapeusis. It may be argued that dehydration of these animals provides an adequate explanation for the high erythrocyte count, yet in other dogs similarly infected by us but without liver therapy this has not proved to be the case (*i. e.*, the erythrocyte count was rapidly decreased). Furthermore, the exudate from the bowel was bloody and not watery as in uncontrolled diarrhea. In spite of the blood picture we have consistent evidence that liver extract introduced intramuscularly failed to control the amebic infection, except perhaps in so far as general body resistance was possibly maintained somewhat longer by the product. These results stand in marked contrast to those obtained by us in oral administration of raw liver, which definitely controlled the infection (as, for example, in dogs 222 and 223, in which liver by mouth prevented the infection from "getting out of hand"). We may conclude, therefore, that the fraction of raw liver which is efficacious as an amebostatic agent, is either different from that which prevents erythropoiesis, or that its amebostatic action is inhibited when it is introduced intramuscularly.

Effect of Myocardial Destructive Agents on Creatine Content of the Rabbit's Heart.

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For a further experimental study of the creatine changes in damaged heart muscle we have employed the method of Fleischer and Loeb¹ for the production of myocarditis. This involves the intravenous administration of caffeine or sparteine, followed in a few minutes by adrenalin intravenously.

Caffeine-sodio-benzoate (25 mg./kilo) was injected intravenously in the marginal ear vein of rabbits; after a wait of 3 minutes this was followed by the adrenalin (0.1 mg./kilo), given very slowly, taking 3 or 4 minutes for the injection. Some of the animals died within a few minutes. Of those that survived, 15-20% were found dead the following morning, with fluid in their pleural and pericardial cavities. A few more succumbed in the following days, but most of those alive 24 hours after the injection survived until sacrificed.

In a series of 69 rabbits the chemical changes have been followed at time intervals up to 31 days. The creatine content of the heart was determined by Myer's modification² of the method of Rose, Helmer and Chanutin; these values were reduced to terms of dried weight by drying one sample to a constant weight at 105°C. and calculating the percentage of solids.

Normal Values. The total creatine value for the normal rabbit determined on 25 comparable hearts, *i. e.*, from animals 1200-1500 gm., was found to be 165 mg. % with a standard deviation of 14.7. The dried weight averaged 21.7% of the moist weight.

Spontaneous Deaths. In the 6 animals that died immediately after injection, the creatine values averaged 169 mg. %. Eleven rabbits died spontaneously in the 18-24 hour interval. The creatine content ranged from 68 to 91 mg. %, averaging 83 mg. %. The edema present was shown in the lowered total solids, which averaged 18.55%, with limits of 17.62% to 20.20%.

Later spontaneous deaths were, one at 4 days: creatine 55 mg. %, solids 15.88%; one at 13 days: creatine 125 mg. %, solids 20.28%; and one at 20 days: creatine 110 mg. %, solids 20.14%.

¹ Fleisher, M. S., and Loeb, L., *Arch. Int. Med.*, 1909, **3**, 78.

² Seecof, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

Preagonal Sacrifice. In these animals, with death seeming imminent at periods from 1 to 9 hours, the creatine values were normal or slightly low, but all were definitely lower than corresponding animals sacrificed while showing no abnormal signs, as described in the next paragraph.

Sacrificed Animals. The myocardial creatine content (Table I) was elevated to an average of 193 mg. % in the period from 1 to 12 hours after injection. In these, the solids which averaged 22.4% were uniformly higher than the normal. The values found in the animals sacrificed after longer periods of 1 to 31 days averaged 132 mg. % (20.5% solids) with a definite tendency toward more normal values as time went on.

TABLE I.
Creatine Changes in Experimental Myocarditis.

No. Animals	Exp. Condition	Time	Death	Fresh Muscle Creatine mg. % Wet Weight Aver.	Solids %
25	Controls	Instant	Sacrificed	165 mg. % ±14.7 S.D.	21.6
11	Caffeine + Adrenalin	18-24 hr.	Spontaneous	83	18.6
3	" "	4-20 days	Spontaneous, occurring later	89	18.8
14	" "	1-31 "	Sacrificed	132	20.5
8	" "	1-9 hr.	" (preag.)	159	22.7
13	" "	1-12 "	"	193	22.4
2	Adrenalin only	12 "	"	223	23.2
3	" "	24 "	"	136	20.7
2	" "	48 "	"	126	20.0

Adrenalin alone, so far as studied up to 48 hours, seemed to produce a cycle of myocardial creatine changes which paralleled the course of chemical events produced by the combination of caffeine and adrenalin.

This method of producing myocardial damage seems to have a consistent influence on the myocardial creatine. In the first 12 hours the creatine is increased approximately 20-25% above normal. After that time the values begin to drop. It is suggested that the drop is related to the amount of myocardial damage. Although the critical point is not sharp, when the values drop to about 2/3 of normal, the animal dies. In other hearts the drop is not so low, and as reparative processes ensue, the creatine content gradually rises toward normal.

8434 P

Creatine Content of Hypertrophied Rabbits' Heart Muscle.*

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In connection with studies on the mechanism of cardiac hypertrophy we have analyzed the muscle from 8 hearts of rabbits in which we have experimentally produced cardiac hypertrophy by section of aortic cusps. These animals were sacrificed 14 to 18 weeks after the valve incompetency had been produced. The hearts were divided and the muscle from each ventricle was analyzed for creatine by Myer's modification¹ of the method of Rose, Helmer and Chanutin. These values were reduced to terms of dried weight by drying one sample at 105°C. to a constant weight, thus determining the percentage of solids. The average normal creatine and total solids values for large rabbits such as were used in this series were 180 mg. % and 22.3%.

Eight rabbits with varying grades of hypertrophy from moderate to great degree showed creatine levels which were high in a few instances but dropped to normal and definitely low levels as the amount of hypertrophy increased. There is a slight drop in total solids, especially of the left ventricle muscle indicating a slight increase probably in intracellular fluid content.

In spite of the relatively lowered creatine concentration in the presence of great work hypertrophy the actual creatine content of

TABLE I.
Creatine of Hypertrophied Rabbit Heart Muscle.

HW/BW*	L/R†	—Lt. Vent.—		—Rt. Vent.—	
		Creatine	Solids	Creatine	Solids
			%		%
2.72	1.80	210	22.1	230	22.4
3.26	1.94	174	21.6	193	23.3
3.39	2.16	176	21.4	181	21.3
3.45	2.02	184	21.4	197	22.0
3.58	1.71	182	21.9	219	22.5
4.10	1.69	165	21.1	168	21.6
4.26	1.84	166	22.0	160	22.5
4.68	1.94	159	21.6	152	22.1
	Aver.	177	21.6	186	22.2

*HW/BW = Gm. of heart muscle per kilo of body weight.

†L/R = Ratio of left to right ventricular weight.

* Supported in part by Grant No. 349 from the Committee of Scientific Research of the American Medical Association.

¹ Seeef, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

the individual heart was distinctly elevated. This type of cardiac hypertrophy is comparable to that of hypertension.

8435 P

Creatine Content of Digitalized Normal and Hypertrophied Rabbit Heart Muscle.*

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As a preliminary to a study of the effect of digitalization on the progress of experimental cardiac hypertrophy, we administered digalen and digifoline subcutaneously 0.1 C.U. semi-weekly to one series of 10 rabbits for 4 weeks and to 6 other rabbits for 14 to 18 weeks. These animals were then sacrificed and the hearts divided. Muscle from each ventricle was analyzed for creatine by Myer's modification¹ of the method of Rose, Helmar and Chanutin. These values were reduced to terms of dried weight by drying one sample at 105°C. to constant weight, then determining the percentage of solids.

Large rabbits such as were used in these and subsequent studies on hypertrophy, weighed 2200 to 3200 gm. and showed normal creatine values averaging 180 mg. % with solids averaging 22.3%.

Of the 10 rabbits digitalized for 4 weeks, 3 showed essentially normal creatine and total solids content, while the other 7 showed increased levels, as indicated in Table I. In the series in which digitalis injections were continued for 14 to 18 weeks the creatine percentages were slightly higher while the solids had dropped slightly. Simultaneous studies of the vastus lateralis in all of these rabbits revealed values normal for this muscle creatine (400 mg. %) and solids (24%). Thus the digitalis effect in so far as evidenced by the creatine changes is specific for heart muscle. The digitalization evidently produced no change in the heart weight-body weight or left to right ventricular ratios. The normal ratios are recorded in a preceding report.²

*Supported in part by Grant No. 349 from the Committee of Scientific Research of the American Medical Association.

¹ Seecof, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

² Herrmann, G., Decherd, G., Erhard, P., and Schwab, E. H., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 409.

TABLE I.
Creatine Content of Digitalized Rabbit Heart Muscle.

Digitalis	HW/BW*	L/R†	Left Vent.		Rt. Vent.	
			Creatine	% Solids	Creatine	% Solids
A. Normals						
4 wk.	2.01	1.61	158	22.3	166	23.2
	1.93	1.52	172	22.8	160	23.8
	1.95	1.66	188	22.3	186	23.8
	1.77	1.80	197	22.9	201	24.3
	2.12	1.67	198	21.8	182	22.7
	1.87		202	22.4		
	1.63	1.58	213	23.2	205	23.9
	1.67	1.72	224	22.9	215	24.0
	1.71	1.64	226	23.3	228	25.3
	2.02	1.72	234	22.5	207	23.2
Aver.			201	22.6	195	23.8
14-18 wk.	2.09	1.80	188	21.0	172	23.2
	1.95		197	22.5		
	1.74		199	22.3		
	1.65	1.40	209	21.7	201	22.8
	2.18	2.00	212	22.1	217	23.2
	2.18	1.80	235	22.8	209	23.4
Aver.			206	22.1	200	23.2
B. Hypertrophied						
Small lesion,	2.07	1.88	218	21.2	199	21.9
† hypertrophy	2.07	2.02	227	23.3	217	23.4
	2.21	2.10	249	22.3	214	23.5
	2.30	1.74	270	23.6	256	24.6
	2.41	1.91	230	22.5	224	22.2
Aver.			239	22.5	222	23.1
Large lesion,	2.55	1.76	200	22.3	197	23.4
definite hypertrophy	2.85	1.84	191	22.3	205	23.7
	3.05	1.84	215	22.7	234	22.7
	3.12	1.75	195	21.5	197	21.3
	3.35	1.78	188	22.0	204	23.4
	3.70	2.10	211	22.2	202	23.7
	3.75	2.30	152	22.2	184	22.8
	3.80	2.01	182	22.5	224	22.7
	4.38	1.75	172	22.1	200	22.7
Aver.			189	22.2	205	23.7

*HW/BW = Gm. of heart muscle per kilo of body weight.

†L/R = The ratio of left to right ventricular weights.

While studying the effect of digitalization on the development of cardiac hypertrophy we were afforded an opportunity for determining the possible concomitant chemical changes. Experimental aortic regurgitation was produced in these rabbits, following which, semi-weekly injections of 0.1 C.U. of digalen or digifoline per kilo were maintained for 14 to 18 weeks.

At the end of the experimental digitalization period the animals were sacrificed and the hearts divided and analyzed by our usual methods.

As demonstrated above, digitalization alone has a tendency to increase the cardiac creatine absolutely as well as relatively, while great hypertrophy³ gives relatively a decrease although there is an absolute increase in the actual creatine content of the individual heart.

In 5 rabbits with only small perforations of the aortic sail there was little if any hypertrophy demonstrable by an increase of HW/BW ratio above the normal of 1.972 with its S.D. of 0.299. In these the creatine levels were the highest that we have obtained.

In 9 rabbits with lesions involving at least a whole aortic sail and with conspicuous cardiac hypertrophy there was a normal or elevated creatine percentage and consequently a considerable increase in cardiac creatine content. The digitalization seems to offset the creatine percentage drop of great hypertrophy.

8436 C

Attempted Reversal of Filarial Periodicity in *Dirofilaria immitis*.

E. HAROLD HINMAN.

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Since Manson¹ discovered the phenomenon of filarial periodicity in China numerous investigators have sought to elucidate its mechanism. Mackenzie² was the first to reverse this periodicity by inverting the period of sleep and activity and his success led him to the belief that the sleeping and waking states are primary factors in its production. Manson successfully repeated and varied Mackenzie's experiment and since then a number of investigators have confirmed it, including Low, Manson-Bahr and Walters.^{3, 4} These workers pointed out, on the basis of very careful 2-hour sampling, that true reversal did not occur but an irregular periodicity resulted from the patient's changing his daily routine.

³ Decherd, G., Schwab, E. H., Herrmann, G., and Brown, W. O., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 521.

¹ Manson, P., *The Filaria sanguinis hominis*. London, 1881.

² Mackenzie, S., Haematoehyluria. (Demonstration Pathological Society, London.) *Lancet*, 1881, 707.

³ Low, G. C., Manson-Bahr, P. H., and Walters, A. H., *Lancet*, 1933, 466.

⁴ Low, G. C., Manson-Bahr, P. H., and Walters, A. H., Further observations on filarial periodicity. *Lancet*, 1934, 531.

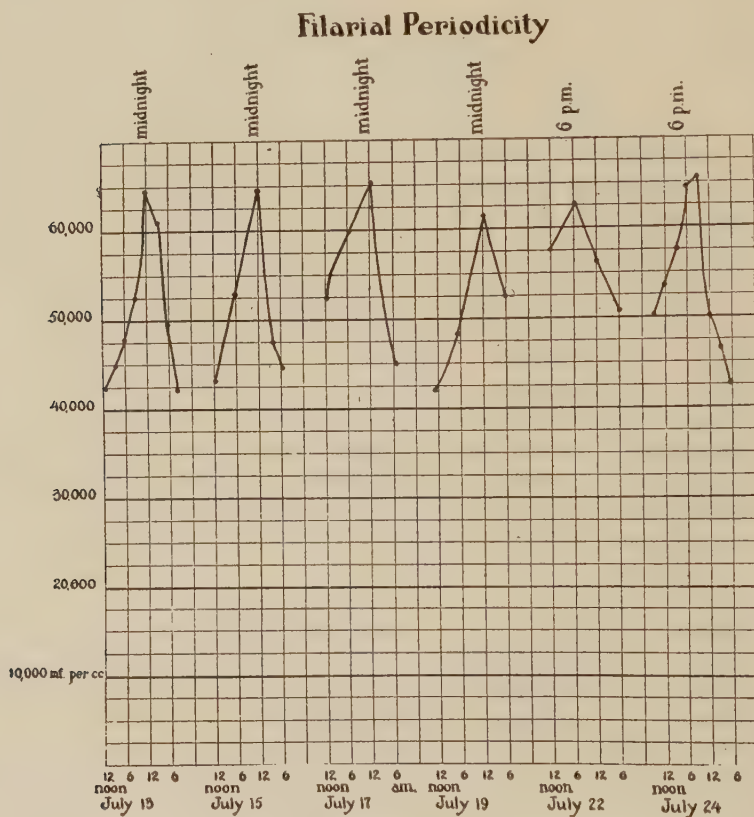
In an endeavor to shed some light on the mechanism of periodicity an attempt was made to reverse the cycle by changing the daily routine of filaria-infected dogs. Two animals were chosen for this experiment, one with a relatively high number of microfilariae (minimum of 40,000 per cu. cm.) and the other with only one-tenth this number. The animals were placed in separate cages, small enough to prevent much exercise, in a basement room without windows but ventilated by a fan system, run continuously throughout the experiment. Filarial counts were always made in duplicate, using 2 calibrated pipettes, which in the case of the dog with the heavy infection removed 8 cu. mm. of blood and in the one with the lighter infection 20 cu. mm. The blood sample was obtained by cutting the margin of the ear of the dog so that the blood ran freely and after duplicate samples were taken, bleeding was immediately stopped by pressure. It is essential that no clotting occur during the securing of the sample. The thick film technique was utilized for staining.

After a normal cycle had been obtained by taking samples every 3 hours for 24 hours, the dogs were kept in darkness and quiet from 6 A. M. to 6 P. M., at which time the lights were turned on. The animals were fed only once daily, at midnight. They were also exercised at this time and during part of the experiment, each animal had the run of a passageway for 6 hours during the night, either 6 P. M. to midnight or midnight to 6 A. M. They were also exercised during the night when blood samples were removed.

It is felt that in this experiment light and feeding habits would probably play the most important part. No means were available to make sure that the animals exercised or slept during the appointed time, but they were given the opportunity to do so. It was believed advisable not to administer hypnotics because of the possibility of their influence on periodicity. Exercise was enforced for short periods during the night when blood samples were taken. It was physically impossible to continue samples every 3 hours over a 2-week period so the intervals were lengthened to 6 hours for a portion of the experiment and alternate days omitted as shown on the graphs.

The experiment was begun on July 13, activity reversed on July 14 and continued through July 24. Thus for 11 days the dogs were kept in darkness during the day, under electric lights at night, fed at midnight and permitted to exercise at night. The influence of exercise would be difficult to evaluate without the use of a treadmill, which was not available. However, experimental dogs kept

in relatively small cages, having no opportunity for real exercise, still exhibit typical periodicity. The quarters in which the dogs were kept had a rather constant, high temperature which approximated 80°F. throughout the experiment, the daily outdoor maximum at this time was 90-95°F.

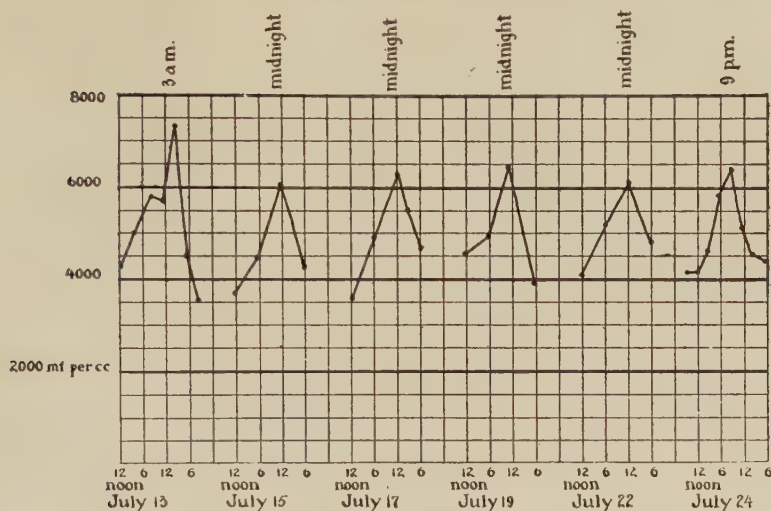


GRAPH 1.

The results for Dog 1 are shown in Graph 1 and those of Dog 2 in Graph 2. At the start each animal showed a typical periodicity with a sharp increase in counts between 6 P. M. and midnight, when the peak was approximated. Between 3 A. M. and 6 A. M. a more marked decrease occurs. At the end of the experiment, Dog 1 showed a maximum at 6-9 P. M. with a decided fall before midnight (see July 22-24). Dog 2 on July 24 showed a relatively high count at 6 P. M. with the maximum at 9 P. M. In this animal there was also a sharp fall before midnight.

These results cannot be taken as a true reversal of periodicity,

Filarial Periodicity



GRAPH 2.

although the daily maximum has been moved forward about 6 hours. However, they do confirm the statement that alteration of daily routine exerts an influence on periodicity.

8437 P

Toxicity of Nembutal for Guinea Pigs.

EMMETT B. CARMICHAEL AND LOUIS C. POSEY.

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The purpose of this investigation was to determine the average lethal dose of nembutal for guinea pigs, that is, the dose which kills 50% of a large group of the animals. All animals used on these experiments were normal and well fed. They were used only once.

The drug was injected intraperitoneally into 510 guinea pigs. The animals were divided into 4 weight-groups and the doses per kilo body weight of guinea pigs varied by 2.5 mg. increments from 42.5 to 67.5 mg. Table I summarizes the results.

The weights of the guinea pigs greatly influenced both the duration of sleep for those animals that recovered and the toxicity for

TABLE I.

Guinea pigs Weight-groups gm.	No. animals used	Dose per kilo mg.	Average lethal dose mg.
200-299	178	47.5-67.5	57.5-60
300-399	158	45 -65	57.5-60
400-599	130	42.5-60	52.5-55
600-799	44	45 -60	45 -47.5

those that died, less of the drug per kilo body weight being required for both of these effects with the heavier animals than with the smaller pigs.

8438 C

Nutritional Value for Planarian Worms of Vitamin Depleted Mammalian Tissues.*

ALICE M. BAHRs AND ROSALIND WULZEN.

From the Department of Zoology, Oregon State College.

The planarian worm has proved itself a useful instrument for demonstrating changes in the composition of mammalian tissues resulting from changing conditions in the animal as a whole. (Bahrs.¹) This research was undertaken to ascertain what the planarian worm could reveal as to modification of the nutritional value of tissues derived from animals fed diets with and without the recognized vitamins.

Guinea pigs and white rats were used to furnish the tissues. The animals received standard basal diets for a month or more. (For diets used, see Table I.) When definite signs of vitamin A deficiency and polyneuritis were apparent in the respective groups of rats, the planarian worm experiments were started. Guinea pigs showed definite signs of scurvy before their tissues were used in the planarian worm experiments. The guinea pigs maintained upon the vitamin D-free diet, of course, appeared entirely normal when their tissues served for the planarian worm diets.

Each group of planarian worms (probably *Planaria agilis*) consisted of 30 newly regenerated worms, all of the same length. Experimental groups were kept in finger bowls of heat-sterilized river

* Aided by a grant from the General Research Council of the Oregon State System of Higher Education.

¹ Bahrs, Alice M., *Physiol. Zool.*, 1931, **4**, 189.

water, and maintained at a constant temperature, 23°C.-24°C. The worms were fed twice per week on thoroughly mashed tissues of freshly killed guinea pigs or rats, taken from groups fed the specified vitamin diets. They always ate the tissues well. Spleen, lung, liver, heart and in one case kidney cortex were the tissues used.

Two types of observations were made upon the worms receiving these various tissues: Weekly individual length measurements, and regular inspection for abnormalities in shape, indicating the presence of dietary disease. It was found that the worms did not remain in a healthy condition on all of the tissue diets, but developed a well-marked symptom complex. When gliding freely, planaria have a very smooth outline, and the first sign of disease was a roughening of this smooth border. This was followed by a deepening in color of the whole worm, and as the disease advanced, the surface of the worm shrivelled and became almost black. Irregular lumps developed which in the worst cases became so large as to bend the worms into abnormal shapes and render movement impossible. Although worms developing this pathological condition continued to eat, the result of disease was shown very early by a decrease in growth. (Table I.) This syndrome is essentially different from the one observed by us in connection with the administration to planaria of liver and egg white in graded amounts. (Wulzen and Bahrs.²)

Reference to Table I shows that lack of any vitamin from the diet of rats and guinea pigs lowered the growth-promoting power, for planarian worms, of all the tissues investigated. What change has occurred in the tissues to make this true is of course unknown. It might be that the weakened condition of animals deprived of vitamins gave rise to metabolic changes which affected the tissues so as to reduce their growth-promoting power. However, the lowered growth-promoting power of tissues from animals on the various vitamin-free diets was not uniform, and also the vitamin D-free guinea pigs were certainly not in a weakened condition, as far as could be observed. Nevertheless, their tissues showed lower growth-promoting power than those of the controls.

The development of disease due to dietary deficiency among the experimental groups of planarian worms was a striking feature of these experiments. Lung and spleen never gave rise to disease, no matter what vitamin was absent from the diets of the animals furnishing the tissues. If guinea pigs had been on the deficient diets for longer periods of time, we have experimental evidence

² Wulzen, Rosalind, and Bahrs, Alice M., *Physiol. Zool.*, 1931, 4, 204.

TABLE I.
Growth-promoting and disease-producing power, for planarian worms, of tissues from rats and guinea pigs fed standard basal diets with or without vitamins A, B, C, and D. All worms in each experimental group (30 worms) were of the same original length, and the experiments were of 4 weeks' duration.

Series	Tissue and Diet	Animal	Orig. Length mm.	Terminal Length mm.	Gain mm.	Gain %	Stand. Dev. and P.E.	Dietary disease in
A.	(A+) spleen†	rat	252	555	303	120	18.538±.132	none
	(A-) "	"	252	465	213	84	15.500±.163	"
	(A+) lung	"	252	486	234	92	16.214±.163	"
	(A-) "	"	252	447	195	77	15.414±.171	"
	(A+) liver	"	252	—	—	—	—	all
	(A-) "	"	252	—	—	—	—	"
	(A+) heart	"	252	357	105	41	10.800±.146	19
	(A-) "	"	252	324	72	28	—	all
B.	(B+) spleen	rat	217	387	170	78	12.928±.146	none
	(B-) "	"	212	323	111	52	10.793±.164	"
	(B+) lung	"	217	406	189	87	13.814±.160	"
	(B-) "	"	212	343	131	61	11.464±.188	"
	(B+) liver	"	217	260	43	19	8.678±.105	20
	(B-) "	"	217	230	16	7	7.730±.106	all
	(B+) heart	"	215	268	53	24	8.933±.139	17
	(B-) "	"	212	278	66	31	9.392±.122	18
C.	(C+) spleen	guinea pig	240	455	215	89	15.178±.172	none
	(C-) "	"	240	429	189	78	14.310±.134	"
	(C+) lung	"	240	401	161	67	13.366±.119	"
	(C-) "	"	240	374	134	55	12.482±.133	"
	(C+) liver	"	210	457	247	117	15.233±.170	"
	(C-) "	"	210	306	96	45	10.400±.164	all
	(C+) heart	"	210	347	137	65	11.586±.105	none
	(C-) "	"	210	332	122	58	11.066±.091	12
	(C+) kidney	"	210	354	144	68	11.800±.101	none
	(C-) "	"	210	253	43	20	8.461±.172	all

D.	(D+)	spleen	guinea pig	240	513	273	113	17.115±.164	none
	(D-)	"	"	240	487	247	102	16.241±.095	"
	(D+)	lung	"	210	396	186	88	14.071±.117	"
	(D-)	"	"	210	338	128	60	12.179±.148	"
	(D+)	liver	"	240	460	220	91	15.370±.164	23
	(D-)	"	"	240	414	174	72	13.793±.215	26
	(D+)	heart	"	210	325	115	54	10.827±.087	none
	(D-)	"	"	210	317	107	50	10.571±.108	" ‡

A. Vitamin A supplied as 5% cod liver oil.* Basal diet: purified casein, 18%; salt mixture No. 185, 4%; yeast, 8%; dextrin, 69%; NaCl, 1%.

B. Vitamin B supplied as 15% of dried yeast.* Basal diet: casein, 18%; salt mixture No. 185, 4%; dextrin, 72%; cod liver oil, 5%; NaCl, 1%.

C. Vitamin C supplied in form of 10 gm. fresh kale per animal daily. Basal diet: ground barley, 40%; bran, 20%; baked milk powder, 26%; CaCO₃, 3%; NaCl, 1%; clarified butter, 10%.

D. Vitamin D supplied in form of 2 drops Viosterol* per animal every other day. Basal diet: yellow corn meal, 76%; wheat gluten, 20%; CaCO₃, 3%; NaCl, 1%. Vitamin C supplied in form of canned tomato juice.

*Yeast, cod liver oil and Viosterol very kindly supplied by Mead Johnson and Company.

†(A+) liver, spleen, etc. = tissue from animal supplied vitamin A.

†(A-) liver, spleen, etc. = tissue from animal devoid of vitamin A.

‡Two worms definitely affected after 6 weeks' feeding.

which leads us to believe that these tissues would also have become disease-producing. Liver, kidney cortex and heart (for one exception, see (D+) heart, Table I) constitute another group, all the tissues of which gave rise to dietary disease in planarian worms, regardless of what vitamin was present or absent, or what type of basal diet was consumed by the animals furnishing the tissues. This last statement requires an important modification. We found that when fresh, green kale served as the source of vitamin C, liver, kidney cortex and heart of the animals so fed were never disease-producing for planarian worms. The nature of this effect of green plant tissue upon the tissues of guinea pigs is under investigation at the present time. In this investigation we are demonstrating, from the condition of the guinea pigs themselves, that the use of vitamin A from certain other sources beside butter does not affect the outcome; neither have other sources of vitamin C beside kale, such as tomato juice, orange juice and ascorbic acid, a protective effect.

The results presented in this paper correlate with those detailed in a recent publication by the authors. (Wulzen and Bahrs.³)

8439 C

Relative Actions of Di-hydro-morphinone Hydrochloride and Morphine Sulphate on the Excised Ureter and Bell's Muscle.*

CHARLES M. GRUBER.

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It has been shown that morphine sulphate, when added to the bath in which an excised pig's ureter is contracting, increases the general tonus and increases the rate and force of the rhythmical contractions of the tissue.¹ These findings have been supported by those of Ockerblad, Carlson and Simon² on the intact human ureter. Recently Krueger and Howes,³ Gruber and Brundage,⁴ and Mitchell

³ Wulzen, Rosalind, and Bahrs, Alice M., *Physiol. Zool.*, 1935, **8**, 457.

* This research was made possible through a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

¹ Gruber, *J. Pharm. and Exp. Therap.*, 1928, **33**, 191.

² Ockerblad, Carlson and Simon, *J. Urol.*, 1935, **33**, 356.

³ Krueger and Howes, *J. Pharm. and Exp. Therap.*, 1934, **51**, 139.

⁴ Gruber and Brundage, *J. Pharm. and Exp. Therap.*, 1935, **53**, 120

and Harned⁵ showed that dilaudid hydrochloride is about 10 times more active than morphine sulphate in bringing about an increase in the general tonus of the intact dog's intestine. Experiments on the excised intestine do not give the same results.⁶ On the excised strips of intestine the actions of these 2 drugs were alike.

The experiments here described were done to determine the relative effects of dilaudid hydrochloride and of morphine sulphate on the longitudinal segments of the excised cat's ureters and Bell's muscle.⁷ The method employed was practically the same as that previously described.⁸ Longitudinal segments of the ureters of cats which had just been killed by a fatal blow on the head were used in all instances. The temperature of the bath, the time interval, etc., were the same as those cited before.

A record was always made of the behavior of the tissue, to serve as a control, before the addition of either drug. Thirty experiments were performed on the excised ureters of 10 cats in which the action of each drug was determined. Fourteen experiments were performed on 10 segments of Bell's muscle in which morphine sulphate and dihydromorphinone hydrochloride were each used.

Dilaudid hydrochloride and morphine sulphate in doses of 25, 50, and 100 mg. were added to the 100 cc. of Tyrode-Locke's solution in which the muscle was immersed. In each case both drugs were tested as to their effects on the same tissue. In some cases morphine was tested first, in others the dilaudid was added first. In either case, the tissue was thoroughly washed with Tyrode-Locke's solution between the additions of the 2 drugs.

Our results show that dilaudid is no more effective than morphine, if as much so, in stimulating the excised ureter of the cat. In many instances 50 and 100 mg. of dilaudid hydrochloride when added to the bath, had no effect in establishing rhythmical contractions, whereas the same amount of morphine sulphate, when added to the bath after thorough washing of the tissue, did establish rhythmical contractions.

Similar results were obtained in the experiments in which Bell's muscle was used.

Because of the frequency with which dilaudid failed to establish

⁵ Mitchell and Harned, *J. Pharm. and Exp. Therap.*, 1935, **53**, 331.

⁶ Gruber, Brundage, DeNote, and Heiligmann, *J. Pharm. and Exp. Therap.*, in press.

⁷ Bell, *Medico-Chirurgical Trans.*, London, 1812, **3**, 171.

⁸ Gruber, *J. Pharm. and Exp. Therap.*, in press.

rhythmical contractions in cases in which morphine sulphate did establish them, it appears to us that dilaudid is not as active as is morphine in establishing and stimulating rhythmical contractions in the excised longitudinal segments of the ureter and of Bell's muscle of the cat.

8440 P

Relation Between Basal Metabolism and Minute Volume of Circulation.

ARTHUR C. KERKHOF. (Introduced by George Fahr.)

From the Department of Medicine, University of Minnesota, Minneapolis.

One of the main functions of the circulation is the transportation of oxygen. *A priori*, reasoning would indicate that there is a close correlation between oxygen consumption and rate of oxygen transportation. Grollman¹ expressed this supposed relationship in his "cardiac index" which correlates minute volume at rest with square meters of body surface. He defines "cardiac index" as the minute volume per square meter of body surface. In a large series of determinations on normal individuals, he found that his "cardiac index" was 2.2 liters per square meter of body surface. The standard deviation in this series of determinations was 0.3. Grollman's cardiac index does not take into account the variability in the basal metabolic rate of normals. As we know, this rate varies —15 to +15 in any large series of normal cases. Believing that a better cardiac index could be obtained by taking into consideration the basal metabolic rate, we have constructed a formula which fits the determination somewhat better than the Grollman index. Our formula which was developed from our own data is minute volume = $2.2A \left(\frac{100 + \text{BMR}}{100} \right)$ where A = the body surface in square meters and BMR is the basal metabolic rate expressed in the usual way. In other words, we would define cardiac index as the minute volume per square meter body surface at 0% basal metabolic rate. When this formula is used to calculate the minute volume, it is found that the determined minute volume will differ only very slightly from the calculated minute volume. In our work we did not confine ourselves solely to normal persons. We used not only a

¹ Grollman, *The Cardiac Output of Man in Health and Disease*. Charles S. Thomas, 1932.

TABLE I.
Determinations of Minute Volume and Cardiac Index.

Date	Case	B.M.R. %	Body sur- face m. ²	Minute vol., liters	Cardiac Index (Minute vol. in L. per m ² body surface at 0% B.M.R.)	Remarks
Normals.						
	R.C.	-28	1.66	3.2	2.67	
	L.G.	-19	1.49	2.6	2.15	
	H.H.	-15	1.77	3.5	2.32	
	He.Ho.	-16	1.78	3.46	2.30	
	C.J.	-21	1.98	3.59	2.29	
	M.K.	+12	1.95	4.43	2.02	
	L.P.	-13	1.49	2.88	2.22	
	"	+23	1.49	3.93	2.14	(Temp. 105°)
	G.R.	+4	2.63	6.4	2.33	
	G.R.	-1/2	2.63	5.73	2.16	
	R.S.	-5	2.42	5.45	2.36	
	T.V.	-5	1.78	3.46	2.03	
Myxedema Cases.						
2-11-35	C.C.	-37	1.94	2.5	2.01	
2-21-35	"	-19	1.94	3.3	2.09	On thyroid medication
2-26-35	"	-18	1.94	3.7	2.32	" " "
3- 7-35	"	-14	1.90	3.76	2.30	" " "
11-14-35	M.E.	-42	1.76	2.30	2.25	
11-15-35	M.E.	-38	1.76	2.17	1.98	
2-15-35	A.J.	-15	1.73	3.6	2.44	" " "
2-16-35	"	-13	1.73	3.2	2.12	" " "
3- 1-35	"	-10	1.71	3.56	2.31	" " "
3- 4-35	"	-18	1.71	2.8	1.99	" " "
3-25-35	"	-8	1.70	3.37	2.15	" " "
3-28-35	H.K.	-3	1.58	3.24	2.12	" " "
4- 3-35	C.K.	-40	1.83	2.38	2.16	
4-12-35	C.K.	-40	1.83	2.30	2.09	
1-29-35	A.P.	-20	1.93	3.05	1.97	
1-30-35	"	-26	1.93	3.20	2.23	
2-14-35	"	-19	1.83	3.30	2.22	" " "
2-28-35	"	-10	1.83	3.25	1.96	" " "
3- 1-35	"	-12	1.83	3.87	2.39	" " "
3- 6-35	"	+24	1.81	5.36	2.38	On thyroid medication (after adrenalin)
3-19-35	"	+2	1.81	4.07	2.20	On thyroid medication
12- 8-34	A.S.	-35	2.18	2.84	2.00	
12-13-34	"	-35	2.18	2.80	2.00	
12-24-34	"	-19	2.18	3.50	1.98	On thyroid medication
1- 5-35	"	+6	2.13	4.58	2.03	" " "
1-12-35	"	+5	2.14	4.50	2.00	" " "
4-25-35	"	+12	2.09	4.87	2.07	" " "
Hyperthyroid Cases.						
2-23-35	Cl.Cr.	+25	1.72	5.2	2.41	
2-26-35	"	+18	1.72	5.49	2.70	
2-13-35	G.T.	+29	1.44	4.10	2.20	On Lugol's solution
2-14-35	"	+14	1.44	3.40	2.07	" " "
2-15-35	"	+14	1.44	3.80	2.31	" " "

Average Cardiac Index = 2.2 ± 0.17

large number of normals with basal metabolic rates varying from -28 to +12 but also determinations on cases of hyperthyroidism, on cases in which artificial fever had increased the basal meta-

bolic rate during the fever, on myxedema cases before and after treatment, on patients whose basal metabolic rate had been changed by reduction in diet, and on cases in which basal metabolic rate had been increased following adrenalin injections, etc. (Table I.) We excluded all cases of valvular heart disease, of heart failure from any cause, and cases showing anemia. The basal metabolic rates in the cases which could not be called normal varied between -42% and $+29\%$. In our series, the standard deviation was 0.17 and the coefficient of variation was 8%. In other words, the variability has been reduced by one-half from Grollman's¹ findings with his cardiac index. We believe that our formula expresses a law of physiology which has no greater variations than other laws of biology; namely that transportation of oxygen is keyed to oxygen consumption.

8441 P

Transmission of Rabbit Papillomatosis by the Rabbit Tick,
Haemaphysalis leporis palustris.

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Rabbit horns occurring on cottontails and on jack rabbits were described by Seton.¹ He reported specimens collected in Oklahoma, Nebraska, and Colorado. Transmission of these tumors was accomplished by Shope, who described them as infectious papilloma. Shope further showed that the causative agent is to be classed as a filtrable virus,² and succeeded in transmitting tumors to domestic rabbits.³

Our first attempts to transmit the infection to domestic rabbits were unsuccessful and we were unable to demonstrate any intracellular structures which might be termed inclusion bodies. Subsequently we succeeded in transmitting the tumors to cottontail rabbits,⁴ domestic rabbits,⁵ and to snowshoe hares.⁶ More recently Beard and

¹ Seton, E. T., *Life Histories of Northern Animals*, Vol. I. Charles Scribners' Sons, New York, 1909, p. 672.

² Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

³ Shope, R. E., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 830.

⁴ Green, R. G., and Shillinger, J. E., *Reports Minnesota Wildlife Disease Investigation*, February, 1934, **1**, 13, 14. Mimeographed U. S. Dept. Agriculture.

⁵ *Ibid.*, July, 1934, **60**, 61.

Rous have established the experimental infection in snowshoe hares and in jack rabbits.⁷

We have observed many cases of the natural infection, and in Minnesota have found the condition to occur in more or less endemic areas. The occurrence of the tumors more conspicuously on the head and neck, and their discrete distribution on almost any part of the body, indicate insect or arthropod transmission.

We have thus far investigated experimental transmission by fleas, wood ticks and rabbit ticks. Six attempts to transmit the tumor by the feeding of fleas have given negative results, as have six trials by the feeding of wood ticks in the nymphal and adult stages. The larval stage of the rabbit tick failed to transmit the infection in one attempt made. A single experiment with the nymphal stage of the rabbit tick gave a positive result.

In this successful experiment 200 flat nymphs were fed in a muslin bag over the ear of a cottontail rabbit carrying an experimental papilloma at the base of the ear. After 24 hours' feeding, 50 ticks attached near the base of the experimental papilloma were removed. The remaining 150 ticks feeding on the ear apart from the horn were allowed to feed for 5 days, and no new horns developed subsequently at the site of feeding. Fifty ticks removed from attachment near the base of the horn were placed in a muslin bag upon one ear of another cottontail rabbit and were allowed to feed for 5 days. Twenty-one days after placement, 2 small horns were observed at the site of tick infestation, and 3 days later 5 additional small tumors were noted in the same locality. Growth continued and they developed a typical infection of rabbit papillomatosis as it occurs in wild rabbits. In conjunction with this experiment, fecal material taken from the infective ticks was scarified into the skin of another cottontail rabbit without causing the production of papillomas.

The rabbit ticks used in this experiment were collected in the Lake Alexander Area, Morrison County, Minnesota. This source practically precludes any possibility of the ticks being naturally infected with the virus of rabbit papillomatosis. Intensive study of this 4,000 acre area over a period of 10 years has failed to reveal any evidence of the virus of papillomatosis. Over 4,000 rabbits have been banded on the area. Recaptures and examinations of wild specimens have been at least 3 times that number. More than

⁶ *Ibid.*, Vol. II, January, 1935, 5, 6.

⁷ Beard, J. W., and Rous, Peyton, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 191.

4,000 rabbits have been collected from the area and have been given minute examination. As the rabbits during the summer time have averaged over 2,000 ticks, there has been ample opportunity to observe the spread of this virus if it were present in the ticks. Many experiments involving the feeding of ticks upon rabbits have been carried out with the collections from the Lake Alexander Area without production of rabbit horns. The possibility of a spontaneous development of the tumors described above would therefore appear to be excluded by the origin of both the experimental animals and the ticks from the Lake Alexander Area.

The development of the experimental horns only on the one ear infested with ticks and the appearance of numerous foci of development within a two-day period and at a time corresponding to the incubation period of the infection, point directly to the tick bites as the source of infection.

Our successful transmission of rabbit horns by the feeding of ticks shows only a mechanical transfer, but demonstrates that sufficient virus may be carried by an insect bite to produce successful inoculation. It appears probable that this infection may be likewise transferred by mosquitoes and biting flies.

8442 C

The Vitamin-B and -G Content of Commercial Beer.

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Since beer is being advertised by several commercial companies as a source of vitamins, it is desirable to have quantitative information on its vitamin content. The only published studies on this question were made on European beers more than 8 years ago. Scheunert and Schiebllich¹ and also Southgate² demonstrated vitamins of the B complex in beer and reported further that A and C are very low or absent. On the other hand, Harden and Zilva,³ while agreeing on the absence of C, were unable to find B in the beer which they examined.

¹ Scheunert, A., and Schiebllich, M., *Chemie der Zelle und Gewebe*, 1924, **12**, 45; *Biochem. Z.*, 1927, **186**, 229.

² Southgate, H. W., *Biochem. J.*, 1924, **18**, 769.

³ Harden, A., and Zilva, S., *Biochem. J.*, 1924, **18**, 1129.

In the present study, the B-complex concentration of several commercial beers was determined by the rat-growth method. Other points studied were a comparison of pasteurized and unpasteurized beer, the effect of filtration through paper in removing vitamins, and the relative concentrations of B and G.

Experimental methods. Healthy albino rats, weighing 35 to 45 gm. were weaned when 21 days old and depleted of vitamins of the B complex on the following basal diet:

Purified casein	20%	Agar-agar	5%
Sucrose	12	Hydrogenated cotton seed oil	12
White dextrin	45	Cod liver oil (standardized for vita-	
Complete inorganic salt mixture....	4	mins A and D)	2
and distilled water <i>ad lib.</i>			

A depletion period of 2 weeks on this diet sufficed to stop all growth in rats weaned at 21 days, so that they could be considered free from stored vitamins. The depleted rats were then started on a test period during which one rat of each litter was continued on the basal diet while its littermates were fed the basal diet plus measured daily doses of the test materials. All of the rats had free access to the basal diet and distilled water, except that water was withheld in those cases in which it might restrict the consumption of beer, because of the water content of the beer.

The rats were confined in galvanized wire cages having screen bottoms—2 meshes to the inch—during both the depletion and the test periods. Littermates were confined together during the depletion period, but they were separated into groups—according to litter, sex, and weight—and each rat was isolated from his fellows during the test period. The daily doses of the test materials were fed separately each morning and the amount eaten each day was carefully noted. The rats were inspected daily, especially for symptoms of B or G deficiency, and their weights, taken before the morning feeding, were recorded for each week of the test period.

Five different beers, A, B, C, D, and E, were fed as supplements to the basal diet. Three of these beers, A, B, and C, came from a local brewery, whereas the others, D and E, came from breweries in 2 other cities. The beers C, D, and E are pasteurized beers which were purchased from retailers in the ordinary 12-ounce bottles, and the beers A, C, and D are advertised as a source of vitamins. The beers A and C are the same except that C is pasteurized. The local beers, A, B, and C, were fed at various dose levels from 2.5 cc. to 30 cc. per rat per day, but the other beers, D and E, were fed only at the 10-cc. level.

It was our experience that weanling rats will not consistently drink more than 10 cc. of beer per rat per day, even when they are denied water. In order to feed more than 10 cc., we evaporated the beer at 38°C. either to dryness or to a thin syrup (2/15 the original volume) and fed weighed or measured quantities of the concentrate. The rats would readily consume either the solids or syrup equivalent to 30 cc. of the original beer, but they would not consistently eat more than this amount. Comparisons of the evaporated and unevaporated beers at the same dose-level have demonstrated that no appreciable loss of vitamins of the B complex takes place during the evaporation at 38°C.

TABLE I.
Growth of Rats with Different Beer Supplements.

Supplement	No. of rats	Weaning weight	Depl'n weight	After 4 weeks No. alive	Weekly gain	After 8 weeks No. alive	Weekly gain
		gm.	gm.		gm.		gm.
0.4 gm. dried yeast	8	35	37	8	22.3	8	19.6
No supplement	8	39	40	0	—	—	—
30 cc. A	8	41	44	8	12.7	8	11.5
30 " B	8	40	40	8	11.0	Not tested	
30 " C	8	38	39	8	11.5	" "	
10 " A	8	37	41	8	2.8	1	—
10 " B	4	42	45	2	2.2	0	—
10 " C	4	41	42	2	3.5	1	—
10 " D	4	39	41	4	3.0	1	—
10 " E	4	40	42	2	1.5	0	—

Results. Table I shows the rat growth and survival when no supplement was given, and when 0.4 gm. dried yeast, or the 5 beers at 2 dose-levels were used as supplements. The figures show that with 30 cc. of the beers A, B, and C, the growth is about one-half that obtained with 0.4 gm. dried yeast; and further that there is no significant difference among these 3 beers at this dose level. At the 10-cc. level the beers A, C, and D, which are advertised to contain vitamins, show about 50% more growth than beers B and E, which are not so advertised. This difference is hardly significant in view of the small number of animals which survived more than 4 weeks at this dose-level; the point to be emphasized, we feel, is that there is not more than 50% difference. The pasteurized beer C allows the same growth as the unpasteurized sample A, from which it was prepared, showing that pasteurization does not change the vitamin-B content of beer. At the 2.5- and 7.5-cc. levels, the rats showed slow (1 or 2 gm. per week) and erratic growth in the first 2 weeks, and then rapidly lost weight and died. At the 15-cc. dose level, there was an average weekly gain of about 5 gm., with no significant difference between the various beers.

None of these rats showed any symptoms of G deficiency. At the 30-cc. dose-levels, no symptoms of B deficiency were shown in 8 weeks, and at the 15-cc. level, none appeared in 4 weeks. At the 10-cc. level, polyneuritis (vitamin B deficiency) was observed in all rats which died between the fifth and eighth weeks.

All of the beers fed in this experiment were filtered at the brewery, and were clear and free from suspended yeast particles. In some other studies, unfiltered beer obtained from the brewery, was filtered at the laboratory, either through sintered glass or through filter paper. The sintered glass filtrates showed no appreciable difference in vitamin content from the brewery filtered beer; whereas filtration through paper caused a loss of some B and nearly all of the G from the beer. In one experiment, only 6 of 12 rats receiving 15 cc. of paper-filtered beer survived a 4-weeks' test period; and although these 6 rats showed an average weekly gain of 3.7 gm., they had practically stopped growing at the end of 4 weeks, and all showed the sunken eyes, bald eyelids, bloody noses, and extensive loss of hair characteristic of G deficiency. These symptoms were never observed among the rats receiving beer filtered at the brewery, or through sintered glass. Incidentally, this observation may explain the failure of Harden and Zilva to find evidences of vitamin-B complex in the beer they investigated; for they mention that they took precautions(?) to see that the beer they used was clear and free from suspended yeast particles.

Table II shows the results of an experiment in which a B extract (containing minimal amounts of G) and autoclaved yeast (G with minimal amounts of B) were fed together, separately, and separately as additions to beer supplements. The B extract and autoclaved yeast were prepared according to Guerrant and Dutcher.⁴ That these supplements were satisfactory for the experiment is shown by the results with the first 3 diets: with both B extract and autoclaved yeast, very good growth, 15 gm. per rat per week, was obtained; with the B extract alone, poor growth (3 gm. per week) and definite symptoms of G deficiency after 4 weeks; with the autoclaved yeast, there was fair growth in the first 4 weeks, 7.8 gm. per rat per week, with all rats dying during the second 4 weeks showing polyneuritis.

When these supplements are added to 30 cc. of beer A, it is seen that while the addition of the B extract increases the growth (15.7 gm. per rat per week) to that observed in the positive control, the addition of the autoclaved yeast increases it to only 12.5 gm. This

⁴ Guerrant, N. B., and Dutcher, R. A., *J. Biol. Chem.*, 1932, **98**, 225.

TABLE II.

Supplement	No. of rats	Weaning	Depl'n	After 4 weeks		After 8 weeks	
		weight	weight	No. alive	Weekly gain	No. alive	Weekly gain
per rat per day		gm.	gm.		gm.		gm.
0.4 gm. autoclaved yeast + B extract equivalent to 0.6 gm. yeast	3	39	37	3	15	3	15.9
B extract	3	47	51	3	3.5	3	2.7
Autoclaved yeast	4	41	48	4	7.8	0	—
30 cc. Beer	4	45	50	4	11.5	4	10.1
30 cc. Beer + B extract	4	42	49	4	15.8	4	15.6
30 cc. Beer + autoclaved yeast	4	43	46	4	12.5	4	12.0

indicates that the limiting factor in the growth of the rats with beer supplements is B rather than G, and that beer contains relatively more G than B. This conclusion is supported by the observation previously mentioned, that we never observed symptoms of G deficiency in animals receiving beer supplement (except the paper-filtered beer); although we consistently observed polyneuritis with beer supplements of 10 cc. or less, after 4 weeks on this diet.

These data permit a calculation of the approximate concentration of vitamins B and G of the beer in standard units. Sherman⁵ defines a unit of vitamin B and vitamin G as that amount which, when fed daily under prescribed experimental conditions, induces a growth in standardized rats at a rate averaging 3 gm. per week over a test period of 4 to 8 weeks. Since 30 cc. of beer caused a growth of about 12 gm., and 10 cc. of about 3 gm. per rat per week, one calculates about 0.1 Sherman unit per cc. of beer or about 3 Sherman units per ounce. The least effective beer, E, shows a concentration of about one-half of this. Since the limiting factor in the growth of the rats is B rather than G, these concentrations refer to vitamin B. The concentration of G is considerably greater, probably at least twice that of B. Since beer contains about 7% by weight of solids, it appears that the beer solids contain about one-tenth as much vitamin B as does dried yeast. Expressed in terms of ordinary commercial yeast, a one-half ounce yeast cake is equivalent to 25 ounces of beer in its vitamin-B content. The vitamin-B concentration which we found agrees approximately with that found by Scheunert and Schiebllich¹ in their bottom fermented (Pilsner) beers, but is distinctly less than that of their top fer-

⁵ Sherman, H. C., *Chemistry of Food and Nutrition*, (4th edition), New York, 1932, 389 and 405.

mented (Porter) beer, for which they report that 5 liters of beer for 60 kg. rat promote a growth which is not entirely normal. Our results show the vitamin-B concentration of beer to be approximately the same as that of milk.⁶

These results show that beer is a wholly inadequate source of vitamin B for the rat. When allowed free access to beer, this animal will not drink enough (at 10 cc. per day) to prevent marked symptoms of polyneuritis; if allowed free access to beer solids or concentrates, it will eat only enough (at the equivalent of 30 cc. of beer per day) of these to show about one-half normal growth because of B deficiency. Assuming that the vitamin-B requirement of the human species per unit weight is not less than one-third that of the rat, a 30-pound child eating the equivalent of 1 liter of beer per day, would show marked symptoms of B deficiency, unless this vitamin were supplied from another source; and even at 3 liters of beer per day there would be subnormal growth.

Summary. By the rat growth method, 5 samples of commercial beer, 3 of which were advertised as a source of vitamins, were found to contain from 0.05 to 0.1 Sherman units of vitamin B per cc. of beer. The concentration of vitamin G is greater than that of B. Pasteurization does not change the concentration of these vitamins. Filtration through paper removes practically all of the vitamin G from beer; filtration through sintered glass has no such effect.

At best, beer is a very poor and inadequate source of vitamin B for rats, and probably also for humans.

8443 P

Adrenal Glands in Dogs with High Intestinal Obstruction.

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In high intestinal obstruction in man,¹ in the dog,² and in the monkey³ the essential changes in the blood chemistry are primarily

⁶ Macy, I. G., and Outhouse, J., Graham, A., and Long, M. L., *J. Biol. Chem.*, 1927, **73**, 189; Samuels, L. T., and Koch, F. C., *J. Nutrition*, 1932, **5**, 307.

¹ Haden, R. L., and Orr, T. G., *Bull. Johns Hopkins Hosp.*, 1923, **39**, 26.

² Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, **37**, 365.

³ Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1925, **41**, 107.

a fall in blood chlorides; usually a rise in alkaline reserve and a rise in non-protein nitrogen. The fall in the blood chlorides is not always conditioned by loss of chloride through vomiting.³ The group of conditions associated with hyperazotemia and hypochloremia⁴ bear certain clinical and chemical similarities; loss of weight, asthenia, and marked prostration, decline in blood pressure, rapid pulse, shallow respiration, dehydration and anhydremia.

The mechanism of the nitrogen retention in these conditions is not well understood, but it would appear to be intimately related in a majority of instances to alterations of salt and water metabolism.⁴ Both clinically and biochemically the syndrome of hypochloremia and hyperazotemia resembles adrenal insufficiency in Addison's disease.

The findings recorded were observed in studies to determine whether the adrenal glands are altered morphologically in the hypochloremic state of high intestinal obstruction as a representative type of this syndrome. The literature contains, to our knowledge, no reference to histologic studies of adrenal glands in high intestinal obstruction.*

High intestinal obstruction was induced on a series of 12 dogs by sectioning the duodenum by cautery and closing the ends by inversion. No food was given for 24 hours before operation and none during the experiment. Water was allowed *ad libitum*. The blood for chemical analysis was obtained from the femoral vein the morning before operation and every morning thereafter until the animal was killed or died. Studies were made for NaCl, urea, CO₂ combining power of blood plasma, specific gravity of blood plasma, and red blood cell count. Immediately after death the adrenal glands were removed and placed in 4% formalin in saline; prepared for histologic study by the paraffin method, and stained with hematoxylin and eosin.

Our studies show that within 24 to 48 hours after operation there

⁴ Wohl, M. G., and Brust, Raymond W., *J. Lab. and Clin. Med.*, 1935, **20**, 1170.

* In a paper by George J. Heuer and Wm. D. Andrus, appearing in the *Annals of Surgery*, Oct., 1934, they state: "Further, since the relatively close similarity in postmortem findings in animals dying after bilateral suprarenalectomy and in those dying following the injection of loop toxin was first emphasized by Sweet⁵ this fact has been frequently commented upon." This reference to Dr. Sweet as well as to his other writings⁶ is incorrect, and there is no specific comparison of the two conditions. Dr. Wm. D. Andrus in a personal communication to the senior author on June 14, 1935, acknowledges this error.

⁵ Sweet, J., *Penn. Med. J.*, 1915, **19**, 492.

⁶ Sweet, J., *Arch. Int. Med.*, 1910, **6**, 536.

is not only a fall in blood chlorides, a rise in urea nitrogen and a rise in CO_2 of the blood plasma as already found by other observers,^{1, 2, 3} but also an increase in specific gravity of the blood plasma and an increase in the red blood cells. The histologic changes of the adrenal glands were as follows: the zona glomerulosa was well demarcated from the zona fasciculata; the former presented a "moth eaten" appearance with loss of cell contour and shrunken nuclei, while the latter had a granular cytoplasm apparently devoid of lipid material.

Two dogs received 500 cc. of physiological salt solution intravenously on the day of operation, and 400 cc. every day after operation. The blood urea remained within normal limits, from 10.4 mg. % on the day of operation, to 14 mg. % on the day the dogs were killed; the blood chlorides were slightly higher on the 15th day, when the dogs were killed, from 446 mg. % to 495 mg. %, the CO_2 had risen only to 47.2 from an initial volume % of 44.5 and the red blood cells decreased from 6,510,000 to 4,670,000. The specific gravity of the blood plasma decreased from 1.0263 to 1.0185. Clinically the animals showed no signs of toxicity but showed loss of 2.4 kilos of weight. The adrenal glands showed the zona glomerulosa clearly defined with plump nuclei, its cells being well filled with lipid droplets; zona fasciculata was also completely filled with lipid showing no evidence of depletion.

Two dogs received 400 cc. of physiological salt solution intraperitoneally every other day; the blood urea increased from 20.5 mg. % to 39.4 mg. % on the 14th day, and blood chlorides decreased from 467 mg. % to 274 mg. %. CO_2 increased from 38.1 to 86 volumes %. The specific gravity was not greatly altered. The red blood cells decreased by 1,700,000. Clinically the animals were toxic and died on the 14th day of the experiment. The adrenal glands showed histologically irregular lipid depletion, a picture midway between the so-called "lipoid-exhaustion" of extreme degeneration and the normal adrenal.

It is suggested that the function of the adrenal cortex is in some way interfered with in experimental high intestinal obstruction in animals. While our studies are not as yet conclusive it appears that intravenous administration of salt solution in adequate amounts tends to prevent the toxemia and alteration of NaCl and blood urea as compared with dogs that received insufficient or no salt solution. The adrenal glands in animals receiving sufficient amount of salt, are intact.

Work is now in progress with large doses of Cortin alone and

simultaneous salt solution administration in dogs with high intestinal obstruction. Since burns are chemically characterized by hypochloremia and hyperazotemia we are experimenting with Cortin and salt solution as a detoxifying agent. The results will be reported elsewhere.

8444 C

Differentiation of Ergosterol from Cholesterol.

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Ergosterol and cholesterol are sterols each containing in its molecule 3 benzene rings with the configuration found in phenanthrene, one 5-membered ring with a side chain and one secondary alcohol grouping. Ergosterol, however, has one more carbon atom than cholesterol, one hydrogen in the side chain being replaced by a methyl group, and it also has 3 double bonds instead of one double bond.

The greater degree of unsaturation of ergosterol argues for its greater reactivity. Heilbron and Spring¹ report that ergosterol yields a red color with antimony trichloride, but no reaction takes place with α - and β -dihydroergosterol, with α - and β -ergostenol (tetrahydroergosterol) with allo- α -ergostenol (hexahydroergosterol), or with cholesterol. They also report that the trichloroacetic acid reaction with ergosterol, which is negative with cholesterol, is not given by partially or completely saturated derivatives of ergosterol.

Several other tests reported for ergosterol fail to give responses with cholesterol. Among these we may mention the reaction (1) with chloral hydrate,² (2) with a solution of bromine in chloroform (Tortelli-Jaffé reaction)¹ (3) with concentrated nitric acid or with a solution of mercuric acetate in nitric acid,³ and (4) with a solution of selenium dioxide.⁴ Montignie⁴ and also Callow and

¹ Heilbron, I. M., and Spring, F. S., *Biochem. J.*, 1930, **24**, 133.

² Rosenheim, O., *Biochem. J.*, 1929, **23**, 49.

³ Rosenheim, O., and Callow, R. K., *Biochem. J.*, 1931, **25**, 24.

⁴ Montignie, E., *Bull. Soc. Chem.*, 1932, **51**, 144.

⁵ Callow, R. K., and Rosenheim, O., *J. Chemical Society of London*, 1933, 387.

Rosenheim⁵ state that selenium dioxide is reduced to free selenium not only by ergosterol but also by dihydroergosterol. A test to differentiate ergosterol from cholesterol may be carried out by use of a reagent containing concentrated sulphuric acid with sodium selenite, the sterol being dissolved in chloroform.⁶ The chloroform layer remains free from color in case of ergosterol, while the acid layer changes to dark red brown to light brown with no green fluorescence. In case of cholesterol a deep bluish purple develops in the chloroform layer, while the lower or acid layer is red brown without a display of green fluorescence. The test cannot sharply differentiate ergosterol from cholesterol in mixtures of these 2 sterols, and is therefore not serviceable in detection of small traces of ergosterol in cholesterol.

We now report a method for differentiating ergosterol from cholesterol in chloroform solution by means of a reagent containing 3 volumes of 2% aqueous solution of sodium selenite and 1 volume of concentrated hydrochloric acid. A reagent containing equal volumes of 2% sodium selenite solution and orthophosphoric acid (85%) or one containing 3 volumes of the selenite solution and one volume of concentrated sulphuric acid is also effective. The best results, however, are obtained with the selenite reagent acidified with hydrochloric acid. In alkaline medium the sodium selenite solution does not react.

The chloroform for dissolving the sterol must be free from substances that reduce the selenite to elemental selenium. Non-reactive chloroform may be obtained by washing U.S.P. chloroform 3 times with water in the proportion of one volume of organic solvent to 3 volumes of distilled water.

The reaction is carried out as follows: 2 cc. of a chloroform solution of ergosterol together with 2 cc. of the selenite reagent acidified with hydrochloric acid placed in a test tube are heated on the water bath until the chloroform layer is evaporated. The evaporation takes about a minute. Upon replacing the 2 cc. of chloroform lost, a deep orange to barely discernible yellow color develops in the chloroform layer, the intensity of the color depending upon the concentration of the sterol. The aqueous layer remains colorless. Up to and including 0.2 mg. of ergosterol the orange color predominates, while below that amount the light yellow color develops. The yellow color is well recognizable with 0.05 mg. of ergosterol and is still perceptible with 0.01 mg. of ergosterol.

⁶ Levine, V. E., and Richman, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 832, 833, 1010.

The colors developed in the reaction are partly due to reduced selenium and partly due to the oxidation of the sterol by the selenite. Potassium cyanide reacts with free selenium in neutral or slightly alkaline to form a colorless solution of potassium selenocyanide (KCNSe). We have treated the reaction product obtained from selenite and ergosterol with a solution of potassium cyanide, and have found but slight diminution in the characteristic color. The failure to decolorize may be due partly to the presence of colored product derived from the oxidation of the sterol and partly to the fact that the free selenium may be adsorbed upon the sterol or some of its reaction products so that it may not be free to react with potassium cyanide.

Ergosterol irradiated with the Hanovia mercury lamp at a distance of 30 inches for one hour also reacts. Non-irradiated ergosterol shows a more gradual change of color from deep orange to yellow, whereas the irradiated ergosterol gives a sharp gradation from a definite orange with 1 mg. of the sterol to a slight orange yellow with 0.5 mg. to a definite yellow with quantities ranging from 0.2 mg. to 0.01 mg.

Cholesterol, non-irradiated as well as irradiated under the same conditions as ergosterol, yields no color reaction. Cholic acid, which is related to the sterols in chemical composition, does not react. Carotene is also not reactive. Cerevisterol, which, according to Honeywell and Bills,⁶ has two double bonds, gives a positive reaction, although Callow and Rosenheim find that apocholic acid reduces selenium dioxide. Cerevisterol, which according to Honeywell and Bills,⁷ has two double bonds, gives a positive response. Carotene does not react.

Calciferol, one product isolated from irradiated ergosterol, and possessed of vitamin D potency, responds to the test but not as intensely as irradiated or non-irradiated ergosterol. One milligram of calciferol dissolved in chloroform gives a test comparable to that given by 0.2 mg. of ergosterol. The limit of sensitivity for calciferol is 0.25 mg. Callow and Rosenheim⁵ report that lumisterol reacts with selenium dioxide to liberate selenium.

Ergosterol in chloroform solution also reacts with 5% solution of selenious acid, and with a reagent containing 3 volumes of 2% sodium selenate solution and one volume of concentrated hydrochloric acid. Cholesterol does not react with the above reagents.

The selenite reagent acidified with hydrochloric acid is very useful in the detection of minute quantities of ergosterol in choles-

⁷ Honeywell, E. A., and Bills, C. E., *J. Biol. Chem.*, 1933, **103**, 515.

terol. Ordinary cholesterol, according to Bills,⁸ contains less than 5 parts of ergosterol in 1000 parts or less than 0.5%. We dissolved one gram of cholesterol in 5 cc. of washed chloroform and added 2 cc. of the selenite-hydrochloric acid reagent. A reaction was obtained, which when compared to a series of standards, indicated the presence of 0.25 mg. of ergosterol in the one gram of cholesterol (0.025%). We made up a mixture of 100 mg. of ergosterol-free cholesterol and 0.025 mg. of ergosterol (0.025%) in 5 cc. of chloroform, applied the test and obtained a positive reaction.

Conclusion. A mixture made up in the proportion of three volumes of 2% aqueous solution of sodium selenite and one volume of concentrated hydrochloric acid is useful for the detection of irradiated or non-irradiated ergosterol and calciferol. The reagent is useful in distinguishing ergosterol from cholesterol, since the latter does not react. It is also very serviceable in the detection of small amounts of ergosterol in samples of cholesterol.

8445 C

Further Studies of Sex Skin Reactions in *Macaca Mulatta*.

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It has previously been reported¹ that the sex skin reactions of rhesus macaques, which can be called forth by injection of oestriol, eventually spread over a wide extra-genital area if the injections of this hormone are continued for longer than a month. Minor degrees of this extra-genital oedema can be observed in many untreated cyclic females at certain periods of the year, but the full manifestations possible after prolonged oestriol treatment have not been observed to date in a considerable experience with untreated macaques. In the further study of these phenomena, it was of interest to see whether the results obtained with oestriol could be duplicated with oestrone. Also, in view of the symmetrical character of the sex skin reactions, it was felt desirable to investigate a few relationships of the segmental nerve supply of the affected areas to the reactions occurring therein.

⁸ Bills, C. E., *Physiol. Rev.*, 1935, **15**, 1.

¹ Bachman, C., Collip, J. B., and Selye, H., *Proc. Roy. Soc. Lond.*, 1935, **117**, 16.

Females only were used. Two adult castrates, one adolescent castrate, and 2 adolescent but acyclic intact animals were injected subcutaneously once daily for 48, 48, 74, 74 and 76 days respectively with 500 γ of crystalline-folliculine (Girard) dissolved in corn oil.

A typical experiment will show that the responses previously elicited by oestriol were duplicated by the use of oestrone. Monkey No. 885, a 4275 gm. female castrate, had been used during the original experiments with oestriol, but had received no treatment for 7 months. The genital reaction appeared on the second day after starting oestrone injections, and attained maximum brilliancy on the 16th day. This local reaction, as in most animals which have been repeatedly treated with oestrogenic hormone, was featured more by coloration than by oedema. An analogous situation exists in nature, where the initial genital oedema which characterizes the sex skin phenomena of pubertal females gradually gives way to an erythematous type of reaction during the menstrual cycles of maturity. After the 25th day, the genital color of Monkey No. 885 varied from day to day, but tended gradually to fade in spite of continued treatment. By the end of the injections on the 48th day, it had almost lost its red shade. Meanwhile, and from the 20th day onward, the pachydermatous oedema of the extra-genital reaction gradually spread over hips, thighs, legs, back and brow in this order, and was still slowly spreading at the end of the experiment. Eight days after the injections were stopped, vaginal bleeding occurred and lasted for 3 days.

Except for individual variations, the responses in the remaining animals were analogous. The second adult castrate was an animal which had also been used during the former experiments with oestriol; its reactions in both experiments were not as maximal as in Monkey 885. Since a sub-maximal result was further observed in one of the 3 adolescents, none of which had previously received oestrogenic hormone treatment, these deviations can probably be ascribed to individual variations of response rather than to qualitative differences in the activity of oestrone and oestriol, or to effects of castration upon the actions of these hormones.

For the investigation of nervous factors in the sex skin reactions, both of the adult castrates used above, and a young male, were utilized. In one of the females, a segment of skin from a reactive area on the thigh was transplanted to the abdomen by means of a 2-stage pedicle graft operation. In spite of the severance of all previous segmental nerve supply, the graft reacted as usual to oestrogenic hormone in its new location. In the second female, the

reverse procedure was applied, that is, transplantation of a piece of skin from a non-reactive area on the abdomen to a denuded site within the genital area; treatment in this animal left the transplant a pale island of skin in brilliantly colored surroundings.

In the male, the left abdominal sympathetic trunk was severed below the fifth lumbar ganglion. Treatment with oestrogenic hormone elicited a temporary but definitely greater degree of swelling in the left scrotal area than in the corresponding area on the opposite side. This was soon obscured, however, by the increasing oedema of the genital reaction as a whole. A complementary type of experiment has already been recorded by Zuckerman,² who was able to inhibit the genital response by section of the sacral nerves in the cauda equina.

Summary. In the sex skin reactions of *M. mulatta* there are no qualitative differences of response to the keto- and trihydroxy-forms of oestrogenic hormone. Although the skin reactions are to a degree under the influence of the autonomic nervous system, the characteristic phenomena are probably determined by inherent structural peculiarities of the genital skin itself.

8446 C

Oestrogenic Hormone and the Mechanism of Corpus Luteum Formation in the Rabbit.

CARL BACHMAN. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

Hohlweg's experiment,¹ in which corpus luteum formation was induced in immature "40 to 50 gm. rats" by the administration of single large doses of dihydroxyoestrin benzoate (Progynon-B), constitutes the crucial evidence in support of the currently favored view that oestrogenic hormone stimulates the output of luteinizing hormone by the anterior pituitary. Attempts to duplicate Hohlweg's attractive experiment in this laboratory have unfortunately been unsuccessful. Single subcutaneous doses of as much as 2000 γ of crystalline oestrone (Folliculine-Girard) in oil have been given to each of 10 female rats, weighing from 40 to 50 gm.—this weight corresponding to an age of 24 days in our colony. At autopsy one

² Zuckerman, S., *Proc. Roy. Soc. Lond.*, 1935, **118**, 22.

¹ Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

week later the ovaries of these animals were found to be still immature. There was no histologic evidence of granulosa luteinization. The weights of the ovaries (averaging 12.9 mg. per rat) and of the pituitaries were not significantly altered in comparison with those of uninjected control rats. Barring any theoretical difference in the biologic activity of oestrone and dihydroxyoestrin benzoate, the conflicting results may be due to differences in age-weight relationships in the two colonies. In this connection, Selye *et al.*² have already indicated that the effects of massive oestrogenic hormone doses in rats are first observed with regularity at or near puberty, irrespective of the absolute weight of the test animal.

In order to examine the question further the oestric rabbit was thought to be a desirable test object. As the rabbit exhibits no spontaneous ovulatory cycle, adult does of this species which have been well fed and isolated for 3 or more weeks should, in the majority of instances, have ovaries free of functioning corpora lutea and containing numerous growing and mature follicles. A number of does with a known record of litter-bearing were accordingly isolated, and after the lapse of one month, were inspected by laparotomy for the condition of the ovaries. By eliminating any animals exhibiting hemorrhagic follicular atresia, 12 suitable animals with grossly healthy and mature follicles were found for experiment. Allowing 3 days for recovery of pre-operative weight, these animals were given crystalline oestrone in the following manner: Four received 500 γ in corn oil intramuscularly once daily for 8 days; 4 were given 2 intramuscular injections of 4000 γ each in oil at intervals of 3 days; 4 received a single intravenous injection of 2000 γ partly dissolved and partly suspended in a 5% alcohol-saline solution. Biopsy of the uterus and one ovary was performed at the end of 48 hours in one of the second group of animals. The uterus was moist, intensely swollen and cyanotic at this time, and averaged 12 mm. in cross-sectional diameter. Histologically the ovarian medulla and the stroma of the uterine mucosa were intensely engorged, but otherwise there were no exceptional features to be noted in the minute anatomy of the ovarian follicles and interstitial tissue. The remaining animals were examined by laparotomy on the ninth day of the experiment, at which time biopsy specimens of the uterus were taken in half of the animals of each group. The uteri of all animals were still markedly turgescient at this time, but in only one instance was corpus luteum formation observed. In this instance the corpora

² Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1377.

resembled in all characteristics normal corpora of about the fourth day of pregnancy. The uterine mucosa, however, was not progestational in type, but showed hydrops similar to that observed in the "endometrial mole" of Selye, Harlow and McKeown.³ It is believed that this animal may have been accidentally stimulated sexually during handling, this rather than any direct effects of oestrone upon the hypophysis accounting for the occurrence of ovulation.

In order to extend the experiment on a comparable basis with the experiment of Hohlweg as to dose per unit of body weight, 2 additional 2½ kilo oestric does were each given intramuscular injections of 15,000 γ of oestrone in oil (subdivided into convenient but simultaneously administered portions), and reexamined 72 hours later. In neither case had ovulation or corpus luteum formation occurred by that time.

The original group of 12 rabbits were allowed 3 days in which to recover from the second laparotomy, and on the 12th day following the initial oestrone injections were mated with each of several healthy males. All but 2 does copulated with one or more of the bucks. Two days later the does were killed. In all but the 2 does which had refused to copulate there was abundant fresh apical corpus luteum formation, these bodies corresponding both grossly and histologically to normal corpora of comparable age. The progestational development of the uterine mucosa, however, was interfered with in varying degrees, from that of complete inhibition as noted by Hisaw and Leonard,⁴ to that approaching the complex glandular proliferation of the typical reaction.

The weight of the pituitary gland (stalk included) in the 14 rabbits averaged 38 mg., and with one exception did not exceed 42 mg. This exception was noted in one of the 2 animals killed 72 hours after receiving 15,000 γ of oestrone; here the gland weighed 72 mg. The average pituitary weight of 21 non-pregnant adult does of the same stock and weight, untreated with gonadotropic or oestrogenic hormones and killed during the course of acute experiments in this laboratory, was found to be 42 mg.

It thus appears that within the limits of the methods and the dosages here chosen, oestrone does not cause luteinization of the ovarian granulosa of the adult oestric rabbit. Oestrone also has little if any effect upon the pituitary weight of female rabbits which

³ Selye, H., Harlow, C., and McKeown, T., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1253.

⁴ Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1930, **92**, 574.

possess no corpora lutea, or which exhibit corpora of less than 3 or 4 days' maturity. The administration of large doses of oestrone does not appear to interfere with post-coital ovulation and early corpus luteum development in the rabbit, though it may alter in marked manner the anatomic pattern of the coincidental progestational mucosa of the uterus. The undoubted stimulating effect of oestrone upon the anterior pituitary, as shown by the work of Wolfe,⁵ Lane,⁶ Hohlweg,¹ Selye, *et al.*,² and others,⁷⁻¹⁰ might therefore be interpreted—as Selye has in fact already suggested—as the calling forth of pituitary factors which have functions other than that of initiating the luteinization of follicular granulosa.

8447 C

Effects of Extract of Cattle Ant. Pituitary on Endochondral Ossification in Thyroidectomized Young Guinea Pigs.

MARTIN SILBERBERG. (Introduced by Leo Loeb.)

From the Department of Pathology, Dalhousie University, Halifax, N. S.

In former investigations we¹ studied the influence of acid extracts of anterior pituitary gland of cattle on the growth of bone and cartilage in young guinea pigs. We analyzed the endochondral ossification in normal animals as well as the callus formation in guinea pigs in which a bone had been fractured; we could establish under these conditions a growth-promoting effect of the anterior pituitary extract. In about 20% of the cases the growth of both cartilage and bone was stimulated to the same extent, with consequent hypertrophy and hyperplasia of their various cells. However, in the majority of the animals the process of ossification predominated over the formation of cartilage, thus causing an earlier closure of the epiphyseal line than would have taken place otherwise.

⁵ Wolfe, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 757, 1192.

⁶ Lane, E. C., *Am. J. Physiol.*, 1935, **110**, 681.

⁷ Burch, J. C., and Cunningham, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 331.

⁸ Halpern, S. R., and d'Amour, F. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 108.

⁹ Hisaw, F. L., *Am. J. Obs. and Gyn.*, 1935, **29**, 638.

¹⁰ Nelson, W. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 452.

¹ Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1423. Silberberg, M., and Silberberg, R., *Ibid.*, 1935, **33**, 177.

The question arises now as to the mechanism by which the extract exerts its effect on bone and cartilage, in particular, whether the pituitary hormone acts directly on bony and cartilaginous tissues or whether it exerts its effects indirectly by way of the thyroid.

Loeb and his collaborators² had tested the effects of the anterior pituitary extracts in thyroidectomized guinea pigs in order to determine whether certain actions of the extract depend on the changes produced in the thyroid gland or whether the extract affects the various organs directly.

In these investigations we wished, therefore, to study the influence of the extract on the growth of bone and cartilage in thyroidectomized young guinea pigs.

Thirty-six winter and spring guinea pigs, averaging from 120 to 280 gm. in weight were used. Under ether anesthesia both lobes of the thyroid gland were completely removed in 26 animals. Injury of the parathyroids was carefully avoided. Sixteen of these guinea pigs were injected daily with 1 to 1.5 cc. extract for 7, 10, 14, and 21 days. As in the former experiments, we used freshly prepared extracts. Ten additional thyroidectomized guinea pigs were not injected and served as controls. A series of 10 guinea pigs of approximately the same weight and size which had intact thyroids served as further controls, their growth being daily observed.

The animals were kept in the same kind of cages and received the same kind of food. After 7, 10, 14, and 21 days some animals of each series were killed; the long bones were taken out as a whole and sections were prepared in accordance with the method previously described.

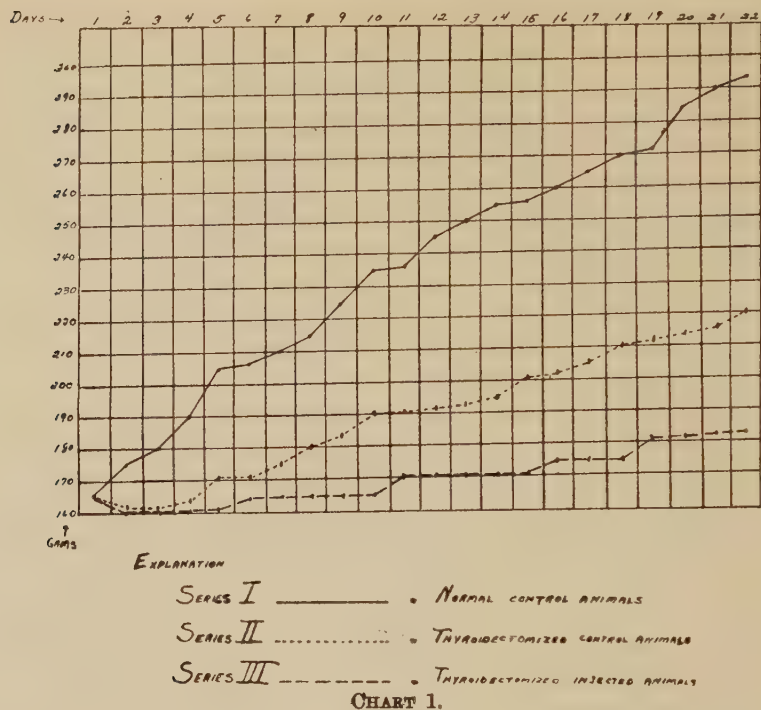
The *normal* guinea pigs gained weight steadily; there was an average rise in 3 weeks from 160 gm. to 295 gm.

The *thyroidectomized* animals lost at first some weight following the operation; after 5 to 7 days they had regained their original weight, and this was followed by a continuous increase of weight up to 220 gm. The endweight in this group was, therefore, lower than that reached by the control animals.

The average endweight of the *thyroidectomized and subsequently injected* guinea pigs was much lower, namely 185 gm. These differences are clearly shown in Chart I, in which the number of days of the experiments represents the abscissae and the weight the ordinate.

There was a parallelism between the difference in weight and the

² Loeb, Leo, *Endocrinology*, 1932, **16**, 129; *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 642; *Ann. Int. Med.*, 1935, **9**, 12.



growth of the animals in these 3 groups. The growth of the thyroidectomized animals was retarded as compared with the normal controls, the whole skeleton remaining considerably smaller; this condition was especially indicated by the length and width of the bony part of skull, thorax and limbs. Still more marked was the retardation in growth of the bones in thyroidectomized, injected animals.

As to the *gross appearance* of the long bones in the thyroidectomized animals, the epiphysis became somewhat clumsy and flatter than in normal guinea pigs and the long bones were shorter.

The decalcification of the bone for the purpose of cutting was accomplished with greater rapidity in the non-injected thyroidectomized animals than in the injected ones, a condition which corresponds to our observations in the experiments on non-thyroidectomized animals.

First Series. In the normal animals the epiphyseal line was always open, the zones of the column-cartilage cells, of the vesicular cartilage and of the calcifying cartilage were well formed and sharply demarcated from one another; the mesenchymal cells of

the chondrophyte were resting and without signs of proliferation or hypertrophy.

Second Series. In thyroidectomized animals, as a rule, the epiphyseal line was open as late as 3 weeks after operation. In comparison with the controls the zone of endochondral ossification was somewhat narrower, while the zone of calcifying cartilage was advancing. In a few cases the amount of calcium within the bony and cartilaginous cells was increased, and in some instances a closure of the epiphyseal line occurred at a later period following thyroidectomy. As to the chondrophyte, from within one to 2 weeks after thyroidectomy the conversion of the indifferent mesenchymal cells into cartilage was more pronounced than in normal animals; the cytoplasm of the cartilage cells was moderately increased and their nuclei were flattened. There was no evidence of hyperplasia or of calcification.

Third Series. In thyroidectomized and injected guinea pigs as early as after 7 injections the growth of the different cell layers of the epiphyseal line was distinctly stimulated. The column-cartilage cells were increased in number and size, mitotic figures being present everywhere and more numerous than normally; the stroma between the cells was distinctly diminished. Likewise, hyperplasia and hypertrophy were noticeable in the vesicular cartilage cells. In addition, disturbances of nutrition seemed to occur, as indicated by the large amounts of calcium taken up by the cells. These finally underwent complete calcification so that the epiphyseal line became considerably narrower and was prematurely closed, an effect more marked and taking place faster than in normal guinea pigs injected with anterior pituitary extracts. Furthermore, an increased growth of the cells adjoining the chondrophyte was seen.

After 10 to 21 injections these phenomena were still more marked; the epiphyseal line was completely calcified; the hyperplasia of the cells of the chondrophyte was more distinct; they had a basophilic appearance and exhibited a strong tendency to calcification.

In contradistinction to the results obtained in normal injected guinea pigs, only in a single case, namely in that one after 14 injections, was the epiphyseal line still open.

Conclusions. In thyroidectomized young guinea pigs injections of acid extract of anterior pituitary gland of cattle cause a hypertrophy and hyperplasia of the different cell layers of the chondrophyte and of the epiphyseal line, with subsequent rapid calcification and premature closure of the latter. We may, therefore, conclude that the extract exerts its growth-promoting effect on cartilage and

bone also in thyroidectomized young guinea pigs. Lack of thyroid gland does not, therefore, prevent the effect of the extract in this respect; on the contrary the phenomena are even more distinct than in normal animals injected with anterior pituitary extract. We may furthermore conclude that anterior pituitary extract exerts its influence on bone and cartilage without the mediation of the thyroid gland.

8448 P

A Comparison of Lipid Composition with Differential Count of the White Blood Cells.

ELDON M. BOYD AND D. J. STEPHENS. (Introduced by W. S. McCann.)

From the Departments of Obstetrics and Gynecology and of Medicine, University of Rochester School of Medicine and Dentistry, and the Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

During the course of a previous investigation, Boyd and Wilson¹ demonstrated that leucocytes from the blood of the umbilical vein and artery contained only about one-half the concentration of certain lipids contained in the white blood cells of adults. It was suggested that the relative lymphocytosis of fetal blood was responsible for the differences observed. The inference was made that in all probability the polymorphonuclear cell contained a higher percentage of lipids than the lymphocyte. The present study was instituted with the purpose of further investigation of this suggestion.

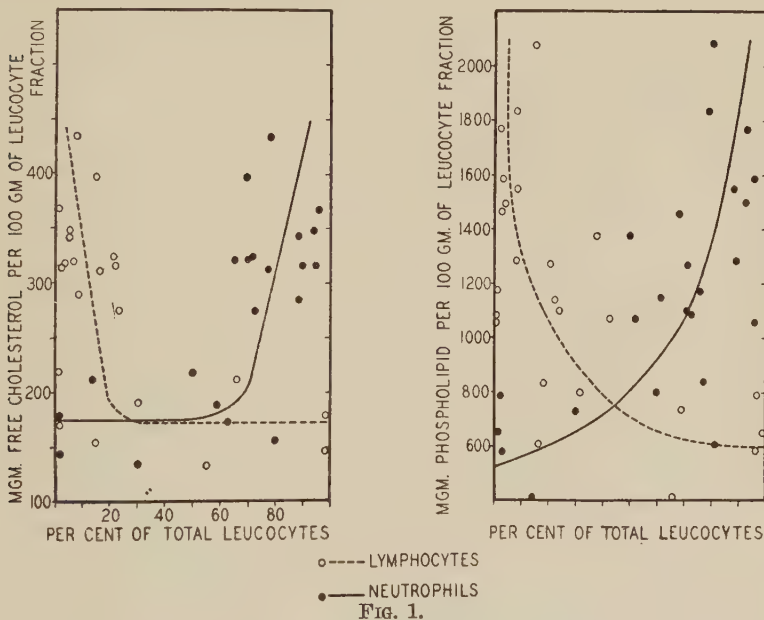
Samples of blood were taken by venapuncture from a group of 25 patients of the Strong Memorial and Rochester Municipal Hospitals. Subjects were selected only with a view to obtaining a wide range of variation in the differential count. The leucocyte fraction ("buffy layer") was isolated and analyzed for its lipid content by a method previously described.² Platelets, the lipid content of which is not known, were included in the leucocyte fraction, but comprised only a small part of the total volume of the buffy layer. The following lipid values were determined: total lipid, neutral fat, total fatty acids, total cholesterol, ester cholesterol, free cholesterol and phospholipid. Lipid values were expressed in milligrams per 100 gm. of the leucocyte fraction, rather than in terms of a given number

¹ Boyd, Eldon M., and Wilson, Karl M., *J. Clin. Invest.*, 1935, **14**, 7.

² Boyd, Eldon M., *J. Biol. Chem.*, 1935, **110**, 61.

of cells or amount of blood. In each instance, total white blood cell and differential counts were made. By comparing the lipid composition of the leucocyte fraction with the differential leucocyte count, it was anticipated that chemical differences in the white blood cells might be demonstrated.

As might have been expected, none of the 8 lipid fractions showed any correlation with variations in the total white blood cell count. Of the various types of white blood cells differentiated in the leucocyte counts, only the neutrophils and the lymphocytes showed variations over a wide range. Phospholipid and free cholesterol content of the leucocyte fraction were found to vary roughly in proportion to the percentage of neutrophils (Fig. 1). Although there were wide variations in the other lipid values, they exhibited no such apparent correlation with the differential count.



It is apparent from Fig. 1 that the higher values for phospholipid and free cholesterol were encountered in those leucocyte fractions which contained a relatively high percentage of neutrophilic leucocytes and a low percentage of lymphocytes. This observation has been interpreted as indicating that the neutrophils contain relatively large amounts of free cholesterol and phospholipid. If the platelet content of the leucocyte fraction is disregarded, the phospholipid content of the neutrophils may be placed at approximately

1500 mg. per 100 gm., the free cholesterol at approximately 350 mg. per 100 gm. These figures are subject to considerable variation. Similarly the phospholipid content of the lymphocyte appears to be in the neighborhood of 600 mg. %, the free cholesterol about 150 mg. %.

The parallelism between free cholesterol and phospholipid adds another bit of evidence to support the hypothesis that these 2 substances are closely related in the vital economy of the cell. Since phospholipid and free cholesterol vary in amount according to the predominating type of cell, it would appear that, for the most part, these 2 lipids serve in the *structural* make-up of the cells. On the other hand, the remaining lipid fractions are apparently present in varying amount in both types of cells, suggesting that they are transitory or metabolic in nature.

8449 P

Experimental Pulmonary Edema.*

VIRGIL H. MOON AND DAVID R. MORGAN.

From the Department of Pathology, Jefferson Medical College.

The frequent occurrence of pulmonary edema as one of the characteristic pathological findings in experimental shock, and following the shock syndrome in human cases, suggested methods by which it might be reproduced. Widespread congestion and petechial hemorrhages predominate as visible changes in rapidly developing shock while in cases in which the shock syndrome develops more gradually there are few petechiae, and edema is more marked.¹

Hemo-concentration is recognized as a constant feature in shock. It results from the transudation of the plasma through vascular walls. Krogh,² Lewis,³ and Landis⁴ support the generalization

* Supported by the Martin Research Fund.

¹ Moon, V. H., and Kennedy, P. J., Pathology of Shock. *Arch. Path.*, 1932, **14**, 360.

² Krogh, A., Anatomy and Physiology of the Capillaries. 2nd ed. 1929, Yale Univ. Press.

³ Lewis, Thos., Blood vessels of the Human Skin and Their Responses. 1927, Shaw & Sons, London.

⁴ Landis, E. M., Capillary Pressure and Capillary Permeability, *Phys. Rev.*, 1934, **14**, 404. Micro-injection Studies of Capillary Permeability, *Am. J. Physiol.*, 1927, **82**, 217.

that any agent or condition resulting in capillary injury increases the permeability of the capillary walls. Edema of the tissues may result by this mechanism. Moon, *et al.*,⁵ found hemo-concentration ante-mortem, and congestion and edema post-mortem in shock arising from various causes.

Experiments were arranged for producing shock of gradual development and sub-lethal degree. A dog weighing 5.4 kg. was given daily subcutaneous injections of 7.5 mg. of histamine phosphate per kg. of body weight, diluted with saline solution 1:250. During 5 days the red cell count rose gradually from 7,200,000 to 9,400,000 and the hemoglobin increased correspondingly—a hemo-concentration of approximately 30%. The animal was killed and examined. Marked congestion of capillaries and venules in the lungs, liver, kidneys and intestines and marked edema of the lungs were found. The alveolar walls were swollen and there was much free fluid in the alveolar spaces. The perivascular spaces were distended with fluid, rich in albumen and containing numerous erythrocytes.

Similar findings resulted when guinea pigs were given subcutaneous injections of 0.5 mg. of histamine per kg. twice daily.

Gluteal muscle was aseptically excised from a freshly killed normal dog. This was finely minced and suspended in saline solution. Twenty grams of muscle substance was introduced into the peritoneal cavity of a dog weighing 6.5 kg. Within 48 hours the red cell count rose from 6,450,000 to 7,970,000 and the hemoglobin from 95 to 125%, indicating hemo-concentration of about 25%. The dog died of shock within 72 hours. Marked pulmonary edema and congestion as described above, were present.

A dog weighing 4.8 kg. received 4.8 gm. of sodium glycocholate in sterile 20% solution intravenously. He died of circulatory collapse almost immediately. There was marked congestion of the venules and capillaries in all the viscera. This was especially prominent in the lungs. Here there was acute edema and numerous capillary hemorrhages.

Another dog weighing 6.4 kg. was given daily intravenous injections of sodium glycocholate, 0.4 gm. per kg. During 6 days his red cell count rose from 6,550,000 to 8,800,000 and his hemoglobin from 85 to 120%. The postmortem findings were as previously described, and the pulmonary edema was extremely marked.

⁵ Moon, V. H., Changes in Blood Concentration Incident to Shock. *J. Lab. and Clin. Med.*, 1933, **19**, 295. Das Schoecksyndrom. *Deutsch. med. Wchnschr.*, 1934, **44**, 1667. The Shock Syndrome in Medicine and Surgery, *Ann. Int. Med.*, 1935, **8**, 1633.

Various barbiturates produce dilatation of the peripheral vascular structures. In our experience the venules and capillaries are particularly affected. We have seen the characteristic shock syndrome develop in dogs following the intravenous injection of sodium pheno-barbital in a dosage of 0.3 mg. per kg. Postmortem findings in such cases were the same as following shock from other causes. Prolonged intoxication was produced by giving to a dog weighing 7.2 kg., 0.3 gm. per kg. of sodium pheno-barbital, twice daily by mouth. A total of 12.1 gm. was given in 4 days. His red cell count rose from 5,150,000 to 6,950,000, and the hemoglobin from 85% to 120%. He died on the fifth day. There was marked congestion of the serous membranes and intense congestion of the liver, lungs and kidneys. Quantities of reddish frothy fluid flowed freely from the bronchi. This fluid had a specific gravity, 1.028, equal to that of the blood serum. Edema of the lungs was extreme.

Another dog, weighing 6 kg., received 9.0 gm. of sodium pheno-barbital during 11 days. His red cell count rose from 5,300,000 to 7,300,000 accompanied by a corresponding increase in the hemoglobin. Bubbling respiratory rales could be heard at a distance of 10 feet from the animal during the last 3 days of treatment. He died on the twelfth day, and the postmortem findings were identical with those in the previous barbital-treated dog. In addition to these findings there was extensive secondary pneumonia of the type described by Moon⁶ in clinical cases following shock of sublethal degree.

Summary. Pulmonary edema may be produced experimentally by the use of agents which produce injury to capillaries. The shock syndrome occurs with varying degrees of rapidity. Experimental shock of gradual development is accompanied by pulmonary edema. This reproduces the features of pulmonary edema which develop in man following severe intoxications. This type of pulmonary edema leads to terminal pneumonia in both human and experimental cases.

⁶ Moon, V. H., Origin of One Type of Secondary Pneumonia. *Am. J. Path.*, 1933, **9**, 899.

8450 C

Excitability of Slowly Reacting Muscle.

H. A. BLAIR. (Introduced by W. O. Fenn.)

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester.

Much attention has been given recently to the alpha excitability of Lucas¹ in the striated muscle of the frog. This excitability which gives rise to a strength-duration curve which approaches closely to its rheobase only with durations of about 200 m.sec. has been the subject of debate by Rushton,² Lapicque³ and others because its time scale is so long that it does not satisfy such generalizations from experiment as Lapicque's theory of isochronism,⁴ while at the same time the same tissue will give results satisfying these generalizations if a different method of stimulation is adopted. In slowly reacting muscle, similar low excitabilities are measured by any of the usual methods, but, as these correspond with the slowness of the other reactions of the muscle, the validity of their strength-duration curves with long-time scales has not been questioned. For this reason the data on these tissues are well suited to providing an answer to the question as to whether the same excitatory mechanism can be postulated in the slow tissues as in the highly excitable ones such as the frog's sciatic nerve.

It has been shown⁵ that the high excitabilities and the few low excitabilities which have been studied are adequately represented by assuming that the local excitatory process is given by

$$\frac{dp}{dt} = KV - kp \quad (1)$$

where p is the excitatory state, V is the stimulus, and k and K are constants, and that there is an independent and much faster process which appears with respect to the process of Equation (1) to be complete for all but very short stimuli and to raise or lower the threshold from a constant value h to $h \pm \alpha V$ where α is a constant.

¹ Lucas, K., *J. Physiol.*, 1906, **35**, 103; 1907, **36**, 113.

² Rushton, W. A. H., *J. Physiol.*, 1930, **70**, 317; 1931, **72**, 265; 1932, **74**, 231; 1932, **75**, 161, 445.

³ Lapicque, L., *J. Physiol.*, 1931, **73**, 189, 219.

⁴ Lapicque, L., *L'excitabilité en fonction du temps*, Paris, Les Presses Universitaires de France, 1926.

⁵ Blair, H. A., *J. Gen. Physiol.*, 1932, **15**, 709, 731; **16**, 165, 177; 1934, **18**, 125; 1935, **18**, 755; *Am. J. Physiol.*, 1935, **112**, 277.

According to this hypothesis the data of rectangular currents are representable by

$$\log \frac{V}{V-R} = kt + \log \frac{K + ka}{K} \quad (2)$$

and those for condenser discharges by

$$\frac{V}{R} = (crk)^{\frac{1}{crk-1}} \quad (3)$$

R being in either case the rheobase and c and r being the capacity and the resistance, respectively, of the condenser circuit.

Data by Bonnardel and Goudchaux⁶ on the ventral muscle band of the leech provide a means of testing these equations as they include both strength-duration and voltage-capacity curves. Some of the data are assumed by the authors to be mixed curves including more than one excitability because the energies of the stimuli have double minima. These cases are avoided, the simple curves only being considered.

The method of applying these equations has been given previously.⁵ In the upper part of Table I are 3 typical strength-duration curves as measured and as calculated according to Equation (2). These measured values are either single sets or the means of several determinations on the same preparation as indicated in the legend. The constants k and $K + ka/K$ are given also in the legend for each case. It will be observed that Equation (2) fits these examples quite well.

In the lower part of Table I similar examples of voltage-capacity data are given as measured and as calculated according to Equation (3). The calculated values in Set F are slightly low for the larger capacities but as this divergence does not occur in the other curves it is probably not significant.

Since these 6 curves are representative of sets of large numbers of determinations which are quite consistent among themselves it seems possible to conclude that the excitatory process in these muscles is adequately represented by Equation (1) along with the faster process whose presence is shown by the constant $K + ka/K$. With these data this faster process is always complete as is evidenced by the fact that Equation (2) is valid even with the shortest stimuli.

It is pointed out by Bonnardel and Goudchaux⁶ (p. 220) that their mixed curves, *i. e.*, the data giving 2 energy minima, indicate that these muscles have a number of excitabilities analogous to the

⁶ Bonnardel, R., and Goudchaux, S., *Arch. internat. physiol.*, 1935, **41**, 201.

TABLE I.
Strength-Duration and Voltage-Capacity Curves of the Ventral Muscle Band of the Leech.

Duration m. sec.	A		B		C	
	Obs. volts	Calc.	Obs. volts	Calc.	Obs. volts	Calc.
∞	10	10	7.5	7.5	12	12
100	17	17	13	13.1	20.5	19.0
80	19.8	20	15	15.1	23	21.8
60	24.3	23.5	18.5	18.3	27	26.1
40	30	31.1	25	24.7	34	34.7
30	37	38.1	29.5	30.5	43	43.3
25					48	49.4
20	48	50.3	41	41.0	54	58.1
15					71	70.7
10	77.5	76.9	67.5	67.5	93	95.3

Capacity mfd.	D		E		F	
	Obs. volts	Calc.	Obs. volts	Calc.	Obs. volts	Calc.
∞	9.5	9.5	6.5	6.5	7	7
9.5	19.5	20.1	11.5	10.4	12.4	10.7
5	27	27.1	13	13.4	15.3	13.5
3.5	31.2	32.8	18	18.0	19.7	17.8
2.5	38	40.3	22	21.6	22.5	21.2
2.0	43.5	46.5	27	26.4	27.3	24.4
1.5	56	57.0	37	36.9	37.3	35.5
1.0	74.2	76.5	50.5	49.7	48.6	47.0
0.8			60	62.1	56.4	55.6
0.7	97.5	99.5				
0.6			74	73.8	69.2	69.3
0.5			85	85.3	79.4	80.2
0.4					94.5	96.9

A. Observed voltages: the means of determinations 4 and 5, p. 216.⁶

$$k = 8.30; \log_{10} \frac{K + k_a}{K} = 0.024.$$

B. Observed voltages: the first set of the experiment of June 8, p. 212.

$$k = 8.09; \log_{10} \frac{K + k_a}{K} = 0.016.$$

C. Observed voltages: the fourth set of the experiment of May 25, p. 212.

$$k = 9.57; \log_{10} \frac{K + k_a}{K} = 0.017.$$

D. Observed voltages: the means of the first and second sets, p. 206. $k = 14.0$.

E. Observed voltages: the third set, p. 207. $k = 14.95$.

F. Observed voltages: the means of all sets, p. 208. $k = 17.7$.

The resistance of the condenser circuit, $r = 13,000$ ohms.

Ag-AgCl wire electrodes were used in all cases, the preparation being suspended in air at room temperature, 15°-24° C.

Lucas excitabilities in the skeletal muscles of frogs and toads. The writer has shown recently⁷ that the energy minimum for condenser stimuli is at $e = 2.718$ rheobases and that at this point $crk = 1$ (Equation 3). Therefore k can be evaluated, approximately, without any further data than the minimal capacity and the resistance.

⁷ Blair, H. A., *J. Gen. Physiol.*, 1936, in press.

Applying this to the mixed condenser curves (p. 219) of Bonnardel and Goudchaux⁶ the upper minimum is with a capacity of about 1 mfd. Therefore $k = 1/1 \times 10^{-6} \times 13 \times 10^8 = 77$,* approximately, which is 5 to 9 times as great as those in Table I.† These excitabilities are not greatly different but with other data (p. 218) 2 curves have their higher minima at about 0.1 and 0.5 mfd., respectively, giving $k = 700$ * and 150, approximately. These values are considerably greater than those in Table I but the data are not very extensive and their validity is not so certain as that of the others (p. 219, data above).

It is concluded from these considerations that the excitatory process in the slowly reacting muscle of the leech is of the same type as that occurring in highly excitable tissue⁵ and that while the excitability as usually determined is about 10 there are others considerably greater. It is not established that these higher excitabilities are from nerves but it does not seem likely that the muscle itself would show such a range with wire electrodes.

8451 C

Parabiotic Twins as a Means of Determining Cellular Individuality.*

M. R. IRWIN AND R. T. HILL.†

From the Department of Genetics, University of Wisconsin.

It has been shown¹ that a division of species-specific substances of the erythrocytes of Pearlneck (*Spilopelia chinensis*), not present in those of Ring dove (*Streptopelia risoria*), is found in the progeny of male hybrids backcrossed to Ring dove. Individual differences in the cells of these backcross birds make reasonable the conclusion that many different heritable cellular components distinguish Pearlneck from Ring dove. It seemed opportune to test whether or not

* Condenser chronaxie is $2/k = 0.026$ sec. and 0.0028 sec., respectively.

† The lower minima in these mixed curves are not well defined but it is indicated that they correspond to excitabilities like those of the simple curves in Table I.

* Paper No. 196 from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin. This work was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council. (Grant administered by Professor L. J. Cole.)

† Formerly Fellow of the National Research Council.

¹ Irwin, M. R., and Cole, L. J., *J. Exp. Zool.*, in press.

a successful "take" of parabionts in backcross hybrids depends, at least in part, upon the relative number of such Pearlneck substances in one member of the pair as contrasted with their absence in the other. The appearance of antibodies would presumably be due to reciprocal immunization, since to date no isoagglutinins have been observed within the Ring dove species nor in the backcross hybrids.

Accordingly, several unions of backcross hybrids, of various ages and in different backcross generations, were made by one of us, (R.T.H.). Kozelka² made parabiotic pairs of very young chicks by a metatarsal union. In our experiments a considerably different technique was used. If the birds to be operated on were under a week old, a thorough chilling in an ice box proved an excellent anesthetic, which reduced blood flow to a minimum, and allowed a rapid recovery. In older birds (young adults) sodium amytal, supplemented with ether, was used as an anesthetic. The operative procedure herein described was for the older birds, being nearly the same for young ones.

The feathers were plucked from each of the wings which were to be united. A ligature was put around the axillary artery in the proximal axillary region, the vessel being easily visible externally. The ligature was put on in such a way as to make subsequent removal easy. The radius and ulna, and their attendant muscles, were next dissected from the humerus, followed by the removal of skin and muscles from the dorsal side of one wing and the ventral side of the other wing. The periosteum was scraped from the bare humerus. The wings were then overlapped, so the 2 humerus bones were in contact, the distal end of one lying next to the proximal end of the other. The 2 bones were then securely fastened with silk, and the overlying skin sutured with silk. The ligature on the axillary artery was removed, making possible the establishment of a fairly good circulation. Only semi-sterile operative precautions were taken. The birds, obviously not able to fly, managed very well in getting around a cage and obtaining food. The tests to be described were made on a pair which had been parabionts for 64 days.

The birds were bled separately, and reciprocal agglutinations by the serum of each were made with their cells, as shown in Table I. The serum from 510H agglutinated the cells of 532B at a higher dilution (24) than did the serum of 532B with 510H cells in the reciprocal test, although there was definite clumping of the cells of 510H by the serum from 532B. These tests show definitely that there were cellular differences between the 2 birds, and also establish the fact of a blood interchange.

² Kozelka, A. W., *Anat. Rec.*, 1929, **44**, 209.

TABLE I.
Tests for reciprocal immunization of parabionts.

Serum	Absorbed by cells of	First Serum Dilution	Agglutinations of cells of:			
			510H	532B	Pearlneck	Ring dove
510H	—	3	0	4	+4	3
532B	—	3	2	0	±	+
Anti-Pearlneck	Ring dove	180	≡	4	+8	≡
	Pearlneck					
Anti-Ring dove	F ¹ Ring dove	45	4	±	0	+4

Symbols: ≡ = doubtful clumping; ± = very faint trace; + = faint trace. The numbers signify the highest serum dilution (by halves) at which definite agglutination was observed under the microscope (*i. e.*, if 1 = 180; 4 = 1440, and 8 = 23,040).

The reactions with Pearlneck antiserum absorbed by Ring dove cells, and with Ring dove antiserum absorbed by the cells of the hybrid between the 2 species, show that the cells of 532B and 510H were characterized, respectively, by specific Pearlneck and specific Ring dove constituents which were not present in the cells of the co-twin. Further, since the serum from each parabiont agglutinated the cells of both Pearlneck and Ring dove, it would seem that cellular differences were present other than those particular to each of the 2 species.

Summary. Each member of a pair of parabiotic twins developed antibodies against the red cells of the other.

8452 P

Augmentation of Ovary-Stimulating Action of Gonadotropic Preparations.*

ARTHUR A. HELLBAUM. (Introduced by M. F. Guyer.)

From the Department of Zoology, University of Wisconsin.

A "prolan-like" substance in the urines and blood sera of different mammals which has little or no action by itself, but which was augmented or "synergized" by the gonadotropic action of an hypophyseal extract has been reported by Evans, Simpson and Austin.¹

* Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by F. L. Hisaw.

¹ Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

Cole and Hart² and Saunders and Cole³ attributed an analogous activity in the blood sera of pregnant and non-pregnant mares to a "luteinizing" substance resembling that found in the pituitary.

The present paper reports the ability of extracts of liver, thyroid, milk, egg white, and lemon juice, to augment the action of pituitary extracts, and deals with the augmentive interaction of human male urine with various gonad stimulating preparations. Female rats 21 to 22 days old were used as test animals and augmentation was determined by the increased weight of the test rats' ovaries over that of the controls receiving gonadotropic extract alone (Table I).

TABLE I.
Ovarian Weights of Immature Rats Showing Augmentation of Gonadotropic Preparations.

Gonadotropic Extract	Ovary Wt.-Gon. Extr. Alone			Augmenting Extract	Ov. Wt.-Comb. Gon. and Aug. Extr.		
	No. rats	Range mg.	Av. mg.		No. rats	Range mg.	Av. mg.
Unfr.Sh.A.P.	9	16-34	27	Male ur. (200 cc. eq.)	9	81-171	112
" " " "	6	11-13	12	" " "	6	63-135	89
Unfr.Hr.A.P.	3	22-27	26	" " "	3	35-79	57
Sh.-F.S.	8	15-26	22	" " "	8	15-29	23
Sh.-Lut.	5	11-14	13	" " "	5	10-13	12
Sh.A.P.							
(F.S. + Lut.)	5	18-26	21	" " "	5	84-104	91
Preg. Urine	3	35-42	37	" " "	4	32-40	37
Pr. Mare Ser.	3	18-21	20	" " "	4	16-20	18
Unfr.Sh.A.P.	6	11-13	12	Milk (100 cc. eq.)	6	51-124	74
(The same amount of the above A.P. preparation was used in the subsequent combinations.)				Egg white (25 gm. eq.)	3	44-57	51
				Lemon (100 cc. eq.)	3	54-86	67
				Hr. Thyroid (.5 gm.)	3	41-62	54
				Beef Liver (.5 gm.)	5	64-128	91

Unfr.Sh.A.P. = unfractionated sheep anterior pituitary.

Unfr.Hr.A.P. = " horse " "

Sh.-F.S. = follicular stimulating fraction of sheep pituitary.

Sh.-Lut. = luteinizing fraction of sheep pituitary.

Preg. urine = human pregnancy urine.

Pr. Mare Ser. = pregnant mare serum.

The male urine preparation was a tannic acid precipitate subsequently extracted with aqueous pyridine, reprecipitated with acetone, and reextracted with water in a procedure like that described for pregnancy urine by Hellbaum, Fevold and Hisaw.⁴

The male urine preparation produced marked augmentation of

² Cole, H. H., and Hart, G. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 370.

³ Saunders, F. J., and Cole, H. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1477.

⁴ Hellbaum, A. A., Fevold, H. L., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1566.

unfractionated pituitary extracts but failed to augment the follicle-stimulating and luteinizing fractions when these were used alone. However, when the 2 fractions were combined, to simulate the unfractionated extract, the augmentive action of male urine was apparent. The action of gonadotropic preparations of extra-pituitary origin, such as urine of pregnant women and blood serum of pregnant mares, is not augmented by the male urine (Table I).†

The milk, egg, liver, thyroid, and lemon extracts were prepared by dehydrating the raw material with excess acetone and extracting the residues with aqueous pyridine. These preparations were combined with unfractionated pituitary extracts only. From 3 to 6 test rats were used to demonstrate the augmenting activity in each preparation, and the results show that the effects of the unfractionated pituitary extract were markedly augmented in each case.

The augmentive action of male urine appears similar to that of extracts from the other sources. Active preparations of male urine are soluble in water, dilute acids and bases, low concentrations of acetone and alcohol, and are precipitated by tannic acid and by high concentrations of alcohol or acetone. These preparations are stable to boiling, but are destroyed by incineration. They are not effective when given orally.

8453 C

Relative Gonadotropic Augmentive Action of Plasma and Formed Elements from Blood of Cattle.*

L. E. CASIDA.

From the Department of Genetics, University of Wisconsin, Madison.

That the blood of cattle contains a substance which interacts with certain anterior pituitary extracts in a manner which enhances the ovary-stimulating effect of the latter was shown first by Evans,

† Investigations similar to these reported here, involving augmenting activity from the blood of cattle, have been carried on by Dr. L. E. Casida,⁵ with similar results. Likewise, the blood of pregnant and non-pregnant mares, in their reactions with the various gonadotropic preparations, appear identical with those of the human male urine and blood of cattle (unpublished data).

⁵ Casida, L. E., *J. Dairy Science*, 1935, **18**, 430.

* Paper from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 197. Published with the approval of the Director of the Station.

*et al.*¹ An attempt has been made in the present study to determine more specifically the portion of the blood which is responsible for the augmentation.

It has been noted earlier² that there was no apparent correlation between the stages of the reproductive cycle represented by the donor cattle and the augmentive power of their blood serum. These serum samples were decanted from clotted blood. It has since been noted that the amount of augmentation from a standard amount of such serum appears in some cases to be proportionate to the redness of the serum, *i. e.*, the amount of hemolysis that has occurred.

Tests have been made of clear plasma and of the sediment or formed elements which were thrown down by centrifugation of freshly drawn citrated blood. The latter were washed 3 times with approximately equal volumes of physiological saline. Test samples of both plasma and sediment were combined with 50% pyridine-33% acetone soluble sheep pituitary extracts prepared by the method of Fevold, *et al.*³ Seven samples of blood from 4 different animals have been tested in this way (Table I).

TABLE I.
Augmentive Action of Plasma and of Formed Elements from 1.67 cc. Cattle Blood.

Blood Sample	Reproductive State of Donor	Average* Ovarian Weights of rats receiving		
		A.P. alone	A.P. + plasma	A.P. + formed elements
7CA	103 days preg.	22	32	86
28CA	130 " "	29	37	90
11CA	125 " "	38	33	78
832A	Immature bull	49	56	100
FMA	252 days preg.	64	93	118
832B	Immature bull	39	36	105
832C	" "	36	39	121
Total No. Test Rats		20	20	20
Mean Ovarian Wt.		39.9±3.4	46.9±5.5	98.5±5.3

Mean difference—Ovarian Wt. of test animals:

A.P. alone and A.P. + plasma = 7.0 ± 6.5 mg.

A.P. alone and A.P. + formed elements = 58.6 ± 6.3 mg.

A.P. + plasma and A.P. + formed elements = 51.6 ± 7.6 mg.

*Average ovarian weights are based on 3 rats in every case except in the tests of sample 832C in which 2 rats were used.

The difference (51.6 ± 7.6 mg.) in the mean ovarian weights of test rats which received plasma and those which received formed

¹ Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

² Casida, L. E., *J. Dairy Science*, 1935, **18**, 430.

³ Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, **104**, 710.

elements is highly significant. The data leave little doubt that the augmentive power of these blood samples was much greater from the formed elements than from the plasma. The mean ovarian weight of the rats which received plasma in addition to the pituitary extracts was not significantly greater than that of those which received pituitary alone (mean difference 7.0 ± 6.5 mg.). In only one case (FMA) did the plasma show definite indication of an augmentive quality.

Earlier studies² had indicated that the augmentive properties of blood serum were not destroyed by boiling. Because of this degree of heat stability tests were made to determine if the "augmenter" were organic or inorganic in nature. The formed elements were incinerated and tested as above at a dosage of 1.67 cc. blood equivalent. Two samples were tested, each on 3 rats. The average ovarian weights of those rats which received incinerated formed elements in addition to pituitary extract were 14 and 37 mg., whereas for the control groups on pituitary alone, they were 30 and 36 mg. The incinerated elements, therefore, did not show evidence of augmentive ability.

Conclusions. The ovary-stimulating effect of certain pituitary preparations is augmented by the blood of cattle; division of the blood into formed elements and plasma shows that the greater part of the augmentive action is attributable to the formed elements. Further, this activity is due to an organic rather than an inorganic substance.

8454 C

Studies in Hodgkin's Disease. II. Unsuccessful Experiments to Transmit Hodgkin's Disease to Dogs by Intralymphatic Injection.

E. M. CHAPMAN. (Introduced by L. Dienes.)

From the Massachusetts General Hospital, Boston.

The transmission of Hodgkin's disease to animals has so far failed and auto-transplantation in man has also failed. Direct injection of Hodgkin's tissue into the lymphatics has not been tried. This method, applied to dogs, seemed of promise as Hodgkin's disease has been described in dogs.¹

¹ MacMahon, H. E., *Am. J. Path.*, 1934, **10**, 309.

Using the method of Drinker the paw lymphatic of 3 large shepherd dogs was cannulated and fresh saline emulsions of diseased lymph nodes were injected. In 2 dogs the injections were repeated so that in all the material from 5 patients with Hodgkin's disease was used. After this the dogs were observed clinically from 7 months to 3 years and then killed. On necropsy of each dog no evidence of disease resembling Hodgkin's could be found.

8455 P

Uterine Temperature of the Rat; a Measurement of Internal Body Temperatures in Unanesthetized Animals.

JAMES B. HAMILTON.* (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine.

Measurements of internal body temperatures have been obtained in unanesthetized animals after previous implantation of fine thermocouples upon the part to be tested. Prolonged investigation may be carried out with a minimal introduction of error if the animals are permitted 48 hours to recover from the effects of visceral exposure and operative anesthesia which are incident to application of any thermometric device. Seven abdominal organs have been studied in various animals during normal conditions, physiological changes (*i. e.*, uterine temperature during oestral rhythms), hypothermia, and hyperthermia. The details of thermocouple implantation, instruments and results will be described in a later paper.

Physiological Changes in the Uterus. Concomitant measurements of uterine and body (colonic) temperatures revealed that the uterus conformed to the level of body heat during all stages of the oestral cycle in the rat. Two-hour tests made daily previous to mating, throughout gestation, and for 18 days postpartum showed that the pronounced organic changes occurring in pregnancy and the subsequent states of lactation and involution also failed to give differential organic temperatures. Even the severe muscular contractions present during parturition did not disturb the agreement of organic and body temperatures, although measurements were taken of one rat during delivery of a remarkably large litter of 17 young. Neither the injection of theelin in doses large enough to cause

* Now at Albany Medical College, Albany, New York.

marked uterine growth nor ovariectomy with subsequent castration atrophy affected the uniformity of temperatures. Epinephrine and pituitary extracts were used to produce rapid responses, but no change in uterine temperature was observed save when the body temperature exhibited a like and concomitant change.

Changes in General Body Temperature. It was found that in a cold environment the body temperature of the rat could be reduced without the aid of anesthesia as much as 45°F., below its normal level with subsequent spontaneous recovery. In all stages of this extreme drop the uterine and body temperatures continued in unison, regardless of whether the organ was in a highly vascular state as in oestrus or pregnancy, or whether it was atrophied as after ovariectomy. In environmentally produced hyperthermia this similarity of the organic and body degree of heat also persisted, even though the body heat level attained and exceeded the lethal point. Stimulation of the body by drugs to produce a febrile condition and the converse, a drug-produced loss of heat, likewise gave a similar uterine-body degree of heat.

Summary. By means of a new technique internal body temperatures were measured in intact and unanesthetized animals. The uterus of the rat was studied during physiological changes which included all stages of the oestral rhythm, pregnancy, parturition, lactation, involution, rapid uterine growth and castration. Uterine temperature was also observed at body heat levels ranging from 55° to 110°F. The temperature of the uterus was found to conform to the body (colonic) level of heat at all times. These results give no evidence for the many claims of temperature differences among the abdominal organs.

8456 P

The Electrothalamogram.*

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If one picks up the electrical potentials from subcortical ganglia in the hemispheres of the mammalian brain, rhythmic discharges

* Aided by a grant from the American Association for the Advancement of Science.

may be recorded, as were also observed by Gerard, Marshall and Saul.¹ Yet one must bear in mind that the so-called spontaneous activity of the cerebral cortex is also manifested by bursts of more or less rhythmical waves, and that the cortex sends many centrifugal pathways to the subcortical ganglia, particularly to the optic thalamus. Thus the question presents itself as to whether the type of discharge observed on these ganglia corresponds to their own activity, also independently of the cortical influence, or whether it is due to the action of the cortex. In the present series of experiments it was tried to approach this problem in regard to the potentials led off the optic thalamus. The records of these potentials may briefly be called the electrothalamogram.

In order to obtain records of these potentials a metal cylindrical tube was screwed into a small trephine hole (diameter of the hole 7 mm.; center of the hole 8-12 mm. behind the coronal suture, 15-18 mm. laterally to the sagittal suture on the cat's skull.) This tube contained a movable hard rubber cylinder in which the electrodes could be fastened. These were concentric needle electrodes as described by Adrian and Bronk,² or 2 insulated silver wires with bare tips, that were coated with AgCl. The nuclei of the optic thalamus were reached in a depth of 15-19 mm. The potentials were recorded by a Sanborn string galvanometer with a 3-stage amplifier previously described.³ All experiments were performed on cats. Post mortem the site of the electrode tips was always controlled on frontal sections.

The electrothalamogram led off the ventrolateral nuclei of the optic thalamus resembles very closely the electrencephalogram as described by Berger,⁴ Adrian and Matthews,⁵ Bishop and Bartley,⁶ and others in that it shows 2 types of waves: slow waves (length from 90-160 σ) and small, quick waves (length from 10-20 σ). These waves persist in superficial ether anesthesia, in dial sleep, in bulbocapnine catalepsy, although they may be quantitatively changed. They can also be found on curarized animals with arti-

¹ Gerard, R. W., Marshall, W. H., and Saul, L. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1123.

² Adrian and Bronk, *J. Physiol.*, 1929, **67**, 133.

³ Spiegel, E., *Arch. Neurol. and Psych.*, 1934, **31**, 469.

⁴ Berger, H., *Arch. f. Psychiatrie*, 1929, **87**, 527.

⁵ Adrian, E. D., and Matthews, B. H. C., *J. Physiol.*, 1934, **81**, 440.

⁶ Bishop, G. H., and Bartley, S. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 698. For literature and further work on cortical potentials see also Fischer, M. H., and *Pflüger's Arch. Phys.*, 1932, **230**, 161; Kornmüller, A. E., *Fortschr. d. Neurol.*, 1933, **5**, 419.

ficial respiration, and are independent of the rhythm of the respiration. They were missed in control experiments after the death of the animals. The electrothalamogram has also in common with the electroencephalogram that sensory stimuli, such as a pin prick to the foot, may diminish or even abolish the slow waves, while both types of waves persist in the cortical (Bremer⁷) and in the thalamic records, if impulses from the spinal cord and rhombencephalon are cut off by transverse section of the midbrain.

In order to eliminate the influence of corticofugal impulses, the cerebral cortex was exposed, leaving over it only a small bridge of bone with the electrode holder. The lateral surface of the cortex was then destroyed by electrocautery and the thalamic potentials were again recorded. Under these circumstances, the electrothalamogram showed also the 2 types of waves as above described. The inference may be drawn that these waves are due to the activity of the thalamic nuclei. If one considers the electric potentials as an indicator of cellular (neural) activity, the close resemblance of the electroencephalogram and of the electrothalamogram seems therefore to suggest that the activity of the optic thalamus is, at least to a certain degree, of a type similar to that of the cerebral cortex.

8457 P

Foetal Respiration in the Rabbit.

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From the Department of Pharmacology and Experimental Therapeutics and the Department of Obstetrics, Johns Hopkins University.

The occurrence of intrauterine respiratory movements in the foetus has been noted¹⁻⁴ but has received scant study.

The use of the method of Snyder⁵ to inhibit parturition in rabbits enabled us to make direct observations upon the full term foetus in the unopened uterus. Anesthetization of the abdominal

⁷ Bremer, F., *C. R. Soc. belge de biol.*, 1935, **118**, 1235.

¹ Preyer, W., *Specielle Physiologie des Embryo*. Leipzig. Th. Grieben's Verlag, 1885.

² Ahlfeld, F., *Monatschr. f. Geburtsh. u. Gynäk.*, 1905, **21**, 143.

³ Reifferscheid, K., *Deutsche Med.*, 1911, **37i**, 877.

⁴ Klemperer, H. H., *Arch. f. Gynäk.*, 1933, **154**, 108.

⁵ Snyder, F. F., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 1.

and perineal region was accomplished by section of the lumbar spinal cord under novocain. After partial immersion of the animal in a bath of warm (38°) Ringer's solution, laparotomy was performed, and the foetuses were observed through the transparent wall of the intact uterus. A cannula was inserted under local anesthesia into the mother's trachea to permit administration of various gas mixtures. This report is based upon observations in 30 pregnant rabbits, studied from the 28th to the 34th day of gestation.

Foetuses within the intact uterus exhibit spontaneous respiratory movements characterized by rhythmic excursions of the thorax and abdomen. Although shallower, they resemble qualitatively the respiratory movements observed after birth. There is great variation in respiratory activity among different foetuses in the same uterine horn, some may be quiescent, others respiring at independent rates up to 60 per minute. The rate usually increases during the first half-hour of an experiment and thereafter remains fairly constant. In general respiratory activity increases near term with age of the foetus. Foetuses followed 24 to 48 hours after an experiment were found to develop normally. The above described foetal intrauterine respiration was not initiated by the experimental procedure since careful observations through the unopened abdominal wall revealed its presence within the normal unoperated animal.

A number of anesthetic agents—ether, paraldehyde, sodium phenobarbital, and sodium pentobarbital—given to the mother in amounts that did not impair respiration but merely induced light anesthesia were nevertheless found to depress seriously or even to inhibit completely intrauterine respiration in the foetus. Respiration could be stimulated or initiated in such anesthetized foetuses by clamping the umbilical cord.

Anoxemia induced in the mother by administration of a gas mixture containing 4% oxygen and 5% carbon dioxide in nitrogen produced immediate slowing or cessation of foetal respiratory movements though the maternal respiration was greatly stimulated. Upon replacement of the low oxygen mixture by air foetal respiration usually returned to normal, but prolonged or excessive anoxemia caused permanent impairment. We have obtained no evidence of stimulation or initiation of intrauterine respiration by oxygen want.

Carbon dioxide (5% and 7½% in air and in oxygen) administered to the mother for periods up to 15 minutes failed to influence foetal respiration to a significant degree though an occasional increase in rate was noted. Breathing was not initiated in foetuses

which were quiescent before carbon dioxide administration was begun.

Hyperventilation of the mother with the aid of a respiratory pump resulted in slowing of respiration or complete apnea in the foetus. Upon suspension of artificial respiration the foetuses remained for several minutes in a state of respiratory quiescence but subsequently resumed their original rate.

On the basis of these studies we regard the onset of post-natal respiratory activity not as an event initiated abruptly at birth but rather as a transition from the type of respiratory movement discernible during intrauterine life. Our observations do not lend support to the view of Barcroft^{6, 7} that the first breath of a new-born is caused by oxygen want. On the contrary, a diminished supply of oxygen depresses foetal respiration in the rabbit. In agreement with the results of Barcroft the foetus appears to be quite unresponsive to excess of carbon dioxide introduced through the mother. On the other hand a certain minimal carbon dioxide tension seems to be essential for the maintenance of foetal respiratory function.

8458 P

Ineffectiveness of Drugs upon Collateral Coronary Flow.*

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Valid experimental evidence as to whether a drug can improve collateral coronary blood flow to an ischemic area is difficult to obtain. Observation of color changes or comparison of size of infarcts without and with use of a drug are subject to too many contingencies to have a certain value. Observations as to the effects of drugs upon coronary inflow and outflow do not test the response of collateral vessels and ignore the hemodynamic alterations resulting from occlusion of the main branch.

We therefore studied the reliability of 4 other possible criteria of

⁶ Barcroft, J., *Lancet*, 1935, **220**, 647.

⁷ Barcroft, J., XV Internat. Physiol. Congress, Leningrad-Moscow, 1935, 21.

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

changes in collateral flow, *viz.*, (1) changes in rate of blood flow from a peripheral coronary ramus; (2) alterations in mean peripheral coronary pressure; (3) changes in inflow rate at high perfusion pressures before and after use of drugs—the latter being administered during a period when the area was not perfused; and (4) the ability of drugs given by inhalation or intravenously to prevent contractile failure after ligating a ramus or of restoring such contractions in an ischemic area. The first and second of the methods cannot be used as criteria. The third method also proved to be no criterion of changes in collateral flow alone but is useful because the flow changes are a *resultant* of vascular and extravascular factors which modify resistance to flow.

The following results were obtained: 1. Drugs of the theobromine and theophylline group have a very insignificant effect on collateral flow to an ischemic region. 2. The nitrite group cause a slight decrease in coronary resistance within the ischemic area but it appears more probable that this is due to attendant reductions of intraventricular tension rather than to effects of drugs reaching the vessels of an ischemic area. At any event this slightly beneficial action is more than offset by the fact that the driving pressure in larger collateral vessels in the left ventricle is reduced. 3. Pressor drugs such as epinephrine and synephrine consistently increase resistance to flow in the ischemic area. If these drugs exert a dilating vascular action on collateral vessels it is overpowered by greater extravascular pressures in intact hearts.

The fourth method offers conclusive evidence as to whether collateral blood supply increases to a degree to be of functional use. This is ultimately the important question. Our results show that the abolition of contraction about one minute after occlusion of the ramus descendens anterior is not modified in the least by inhalation of oxygen, CO₂, amyl nitrite, nor by therapeutic intravenous doses of various theophylline preparations, nitrites, adenylic acid or epinephrine.

The conclusion is reached that an increase in collateral circulation sufficient to be of functional use cannot be attained by use of vasodilating drugs after complete coronary occlusion.

Effect on Pernicious Anemia of Massive Doses of Parenteral Liver Extract.

FRANKLIN R. MILLER.

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With the increased use of parenteral liver extract for both the treatment of the relapse of pernicious anemia and the maintenance of normal blood levels during remissions of the disease, the questions of dose and the intervals between doses have been shown to be of great importance. Castle¹ states: "The parenteral injection at appropriate intervals of material derived from an amount of animal liver equivalent to the weight of that organ in man will suffice to maintain a patient with pernicious anemia in health for at least a year." The accepted dose for most patients with pernicious anemia to maintain the normal blood level is the material derived from 100 gm. of liver given at intervals of one to 3 weeks.² There is, however, in the literature the record of one case³ maintaining a normal blood level for 120 days after massive doses of potent material; and there are 2 other suggestions^{4, 5} that for several months individuals might maintain normal levels if given massive injections of concentrated material.

We have tried to determine whether patients with pernicious anemia could store the potent substance if given in massive amounts over a short period of time, and use it as their needs required. If a patient with pernicious anemia requires the material from 400 gm. of liver (as parenteral extract) for remission from relapse, and then that from 100 gm. every 2 weeks for the maintenance of a normal blood level, that patient would require the material from 700 gm. of liver for maintenance through 14 weeks. It is conceivable, however, that if this were given in a short period of time, some of the antianemic substance might be broken down, and that part of it might be excreted whole.

We have given massive amounts of potent material in divided

¹ Castle, W. B., *Science*, 1935, **82**, 159.

² Murphy, W. P., *J. Am. Med. Assn.*, 1932, **98**, 1051.

³ Wood, I. J., and Gardner, H., *Med. J. Australia*, 1935, **1**, 543.

⁴ Tochowier, L., *Folia Haematologica*, 1934, **53**, 16.

⁵ Abstract of Lecture Delivered in Vienna by Professor Bence of Budapest, Foreign Letters, *J. Am. Med. Assn.*, 1935, **105**, 1447.

doses to 4 patients and have required that they refrain from taking liver or liver extract in any form during a period of observation following the last of the massive dose. These patients all were proven to have pernicious anemia; all had had one or more definite relapses; all previously had had definite reticulocyte responses to the antianemic substance; all had complete anacidity; 2 had definite neurological signs; and with each, previous clinical observations had shown that if the maintenance dose were not sufficient or the interval between doses were too long, the individual began to relapse. Only one of these patients was in relapse at the start of the experiment.

This patient had an erythrocyte count of 0.69 million and a hemoglobin of 28%.* He received an equivalent of 100 gm. of liver by intravenous injection of extract. Then, over a period of 3 weeks, he received an equivalent of 1650 gm. of liver by intramuscular injection of extract. He left the hospital only partially in remission with blood values of 2.8 million erythrocytes and a hemoglobin of 48%; and 49 days later, his peak count of 5.0 million erythrocytes and 84% hemoglobin was obtained, and he was in complete remission. It was only after 91 days following his last intramuscular injection of extract that he showed signs of relapse as evidenced by a falling off in his erythrocyte count and hemoglobin concentration.

The second patient had a normal erythrocyte count and hemoglobin at the time the massive dose was administered. She was given the equivalent of 1650 gm. of liver by intramuscular injections of extract during a period of 10 days. Her erythrocyte count immediately following the last of the massive dose was 4.6 million and hemoglobin 89%. Fifty-six days later, she showed a peak erythrocyte count of 5.1 million and hemoglobin of 98%. Ninety-eight days following the massive dose, she showed no signs of relapse, and her erythrocyte count was 4.8 million and hemoglobin 95%.

The third patient also was in remission when she received an equivalent of 1500 gm. of liver by intramuscular injection of extract over a period of 10 days. Immediately following the last of the massive dose, her erythrocyte count was 4.6 million and hemoglobin 96%. Her peak count occurred 70 days later, at which time her blood values were 5.4 million erythrocytes and 105% hemoglobin. Eighty-four days after the last of the massive dose of

* Sahli hemoglobinometer, standardized, so that 100% equals 15.6 gm. hemoglobin per 100 cc. of blood.

potent material, she showed no signs of relapse and her erythrocyte count was 5.1 million and hemoglobin 103%.

The fourth patient was also in remission. Her erythrocyte count was 4.8 million and hemoglobin 100%. She was given only a relatively large dose or the equivalent of 265 gm. of liver in one intramuscular injection of extract. After 56 days, she had begun to show mild signs of relapse as evidenced by slight gastro-intestinal symptoms; but the erythrocyte count and hemoglobin level showed very little change.

From these 4 cases, we cannot conclude that all patients with pernicious anemia would be able to store the excess of massive doses of active principle given over a short period of time. Nor can we say that any one of these patients was able to store a calculated amount of the material, because so far the remission of none of them has been as long as if the massive dose were given as small doses over a period of 20 or 30 weeks. However, each of these patients shows evidence of storage of some of the active principle, which storage has enabled each one to maintain normal blood levels for a relatively long period of time.

8460 C

Partial Pressure of Oxygen in Arterial Blood of Patients: Description of an Aerotonometer Method.

FORD K. HICK. (Introduced by R. W. Keeton.)

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The partial pressure of oxygen in the arterial blood has been measured indirectly by interpolation of O_2 content on the oxy-hemoglobin dissociation curve, and by estimation of solubilities of CO and O_2 in the subject's blood (Haldane), and directly by aerotonometry. The latter method has been used by Krogh¹ and by Barcroft and Nagahashi.² In this method of the latter authors some difficulty is experienced in keeping the equilibration pressure constant and in securing repeated samples of the small gas bubble for analysis. The present method avoids these two difficulties.

It has been customary to study anoxemic patients by determining the oxygen saturation of the arterial blood and to assume that the

¹ Krogh, A., and Krogh, M., *Skand. Arch. Physiol.*, 1910, **23**, 179.

² Barcroft, J., and Nagahashi, M., *J. Physiol.*, 1921, **55**, 339.

oxyhemoglobin dissociation curve is shifted only as changes in the pH take place. Barcroft³ has studied the partial pressure of oxygen in a few such cases. However, it seemed that a simple, direct method for measurement of oxygen tension would permit a more exhaustive exploration of this field.

About 20 mil. of blood is drawn under anaerobic conditions from a femoral artery into an oiled syringe containing a bit of oxalate-fluoride and mercury, which facilitates mixing. About 10 mil. is then injected into the tonometer chamber in which 0.1 cc. of alveolar air has been placed by sticking the needle through the rubber tube (Fig. 1). The blood foams and mixture is easy with shaking. The

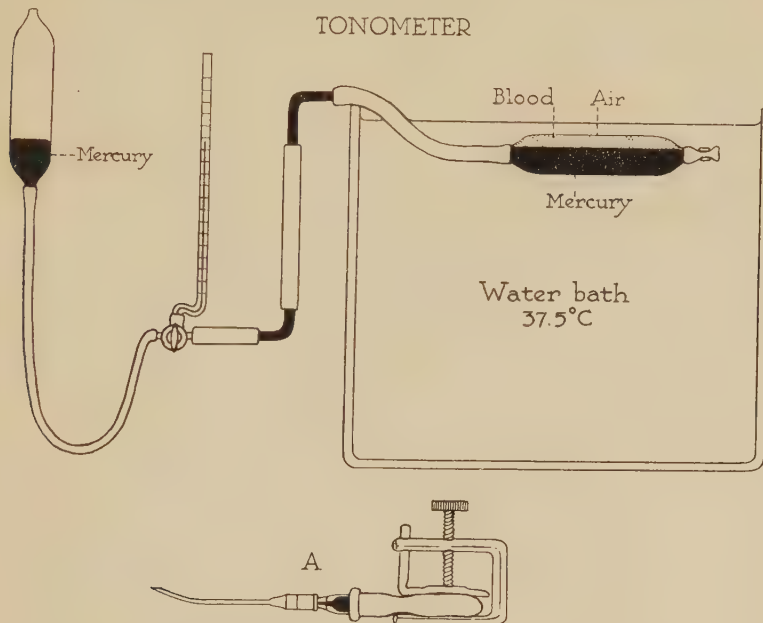


FIG. 1.

The tonometer chamber with its reservoir is connected with a capillary manometer tube. The sampling device is shown below.

remaining blood has its oxygen content and capacity determined in duplicate by standard methods on the Van Slyke manometric gas analysis apparatus.

Samples of the foam are withdrawn after about 10 minutes' shaking at 37.5°C. For this purpose the screw adjusted pipette shown in Fig. 1A is used. The pipette consists of a curved needle with adapter fitted into the bulb of an ordinary medicine dropper and adjusted by a screw clamp. A drop of octyl alcohol is used to

³ Barcroft, J., *Resp. Function of the Blood*, Cambridge, 1914, pp. 226, 282.

moisten the rubber before the whole device is filled with mercury. The tonometer bulb is kept under a known positive pressure of from 10 to 40 mm. by the adjustment of the mercury reservoir. Since the equilibration system is under a positive pressure the pinch cock can be opened for an instant, and while a little blood escapes into the bath, the needle can be inserted to reach the foam. The small samples consisting of about 20 mm. are analyzed serially in the familiar micro-analysis device of Krogh, using air-free normal salt solution or air-free water for filling the burette.

One correction must be made on the analytical results for CO_2 loss in handling the small bubble. It is assumed the arterial blood carries CO_2 at the partial pressure at which that gas is found in the alveolar air. Hence the small bubble should have the same CO_2 content as the patient's alveolar air. The correction is as follows: The observed O_2 content of the small bubble in percent is multiplied by 100% less the difference in the alveolar CO_2 and observed CO_2 percentages.

A small calculation for the whole method is as follows:

M. 11-16-35

Trial	Micro burette reading	Change in length with absorption	% of Gas	Alveolar CO_2 %	Corrected O_2 content
	mm.	mm.			%
I.	96.1				
	92.0	CO_2 4.1	4.27	5.37	
	81.3	O_2 10.7	11.14		11.0
II.	59.0				
	56.5	CO_2 2.5	4.27	5.37	
	50.0	O_2 6.5	11.02		10.9
Mean					10.9(5)

Partial pressure $\text{O}_2 = 10.9(5) \% (B + p - W) = 81.2 \text{ mm.}$

$B = 745 \text{ mm.}$, $p = +40 \text{ mm.}$, and $W = 43 \text{ mm.}$

(p is the positive pressure in the tonometer system. W is the vapor pressure of normal salt solution.)

TABLE I.

Source of blood	Initial pressure of O_2 in gas bag	Partial pressure of O_2 in blood by micro method
Dog	116	117
"	124	123
"	141	142
"	127	127
"	131	130
"	142	138
Human	127	122
"	118	113

The accuracy of the method was checked by experiment on the blood of dogs and men. Samples were equilibrated with gas mix-

tures whose oxygen content was known. Then the oxygen tension in the liquid was determined by this method. The results are shown in Table I.

The determinations on normal people also constitute a check on the accuracy of the method. The results on this group are shown in

TABLE II.

Patient	Oxygen saturation in %	Oxygen partial pressure in mm.
N	97.2	76
"	97.4	81.0
M.C.	95.0	83.5
P.	96.4	80
P.A.	95.6	82
W.	96.2	74
R.	96.8	88
S.	95.4	76
"	95.4	75
Mean	96.1	79.5

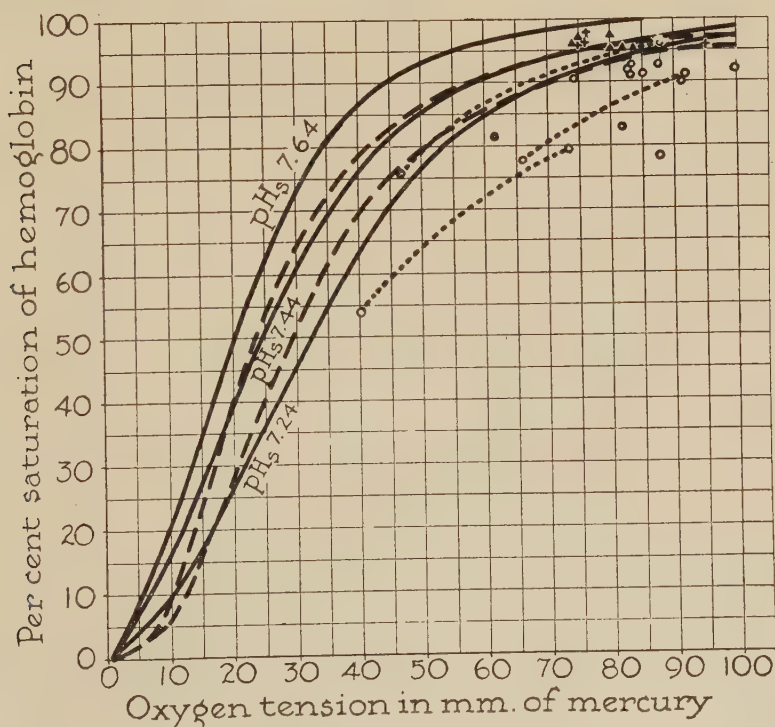


FIG. 2.

The solid line curves are the oxyhemoglobin dissociation curves for the stated pH figures (Henderson). The broken lines are the normal limits of the curves after Barcroft. Normal results from Table II are shown by the triangles and crosses. The circles show an anoxemic situation (Table III). The dotted lines connect successive determinations on the same patient.

Table II. The data from the table is shown on the graph in Fig. 2 in comparison with the normal limits of oxyhemoglobin dissociation curve³ and the curves for various pH figures.^{4, 6} Nine complete studies were carried out on subjects who were hospitalized for non-respiratory conditions and who had no circulatory abnormalities. The O₂ saturation was determined while measurement of the tension of the same sample was in progress. The average O₂ saturation was 96.1%. The average O₂ tension was 79.5 mm.

TABLE III.
Patients with Oxygen Want.

Patient	Arterial oxygen saturation in %	Partial pressure of oxygen
R.	76.2	47
G.	79.4	73
	54.6	40
Gil.	92.2	85
K.	82.2	62
W.E.	91.1	83
C.	93.0	87
	78.0	87
H.	93.0	83.6
S.	92.0	92.6
V.N.	91.0	74
	83.0	82
	92.4	100
Wal.	77.5	66
	90.5	91.5
Sm.	91.9	83

In Table III the presence of an anoxemia in 11 patients with various diseases is shown by an arterial oxygen saturation below 93%. It is apparent from a glance at Fig. 2 that the oxygen tension figures are higher than correspond to the normal limits of the oxyhemoglobin dissociation curve. In patients G. and W. oxygen by nasal catheter was stopped for a time to produce the extreme anoxemia indicated in Table IV. T. W. had an acidosis from a uremia which may have accounted for part of the shift from the expected position of the curve. Patient C., with no anoxemia, shows a normal O₂ tension for the O₂ concentration determined while breathing a low oxygen atmosphere. Observations of such abnormally elevated partial pressures of oxygen are not unique. Henderson⁴ observed it in such unrelated diseases as pernicious anemia, nephritis and myxedema. Litarczek⁵ records it in experimental anemia of rabbits and in anemic patients. Various physico-chemi-

⁴ Henderson, J. J., *Blood*, New Haven, 1928, p. 285.

⁵ Litarczek, G., Aubert, H., and Cosmulesco, I., *C. R. Soc. Biol.*, 1929, **101**, 220.

⁶ Richards, D. W., Jr., and Strauss, M. L., *J. Clin. Invest.*, 1926, **4**, 105.

cal factors control the oxygen combining power of hemoglobin. The K of the Hill-Barcroft equation is known to vary, however, with different bloods at identical temperatures and hydrogen ion concentrations.⁷ These fluctuations may be wide and of importance in pathological states.

Conclusions. 1. A modified aerotonometer method for determining the partial pressure of oxygen can be applied to blood drawn from a human artery and is reasonably accurate. 2. In patients thought to be normal a certain variation obtains. 3. Partial pressure results which are high in relation to the oxygen saturation are commonly seen in the presence of anoxemia.

8461 C

Intravaginal Assay of Urinary Estrin.

WILLIAM R. LYONS AND H. J. TEMPLETON. (Introduced by Herbert M. Evans.)

From the Division of Anatomy, University of California.

Since estrin is not commonly standardized on the basis of its ability to produce behavioristic estrus, but rather by virtue of its growth-promoting influence on the vaginal epithelium, there seems but little need to saturate the entire animal to gain this end. The vaginal epithelium reacts to extremely minute quantities of estrin applied locally,* although Pratt and Smeltzer¹ and Powers, *et al.*,² found their methods of vaginal administration only one-third to one-half as efficacious as the subcutaneous.

We have used the intravaginal method of assaying urinary estrin not only because minute amounts may be detected (even in male urine), but also to avoid the toxic effects produced by parenteral injection of crude extracts. Twenty-four-hour urines were obtained from 4 women† on days 7, 14, 21, and 28 of the menstrual

⁷ Judd, E. S., Snell, A. M., and Hoerner, M. T., *J. A. M. A.*, 1935, **105**, 1653.

*Cornification of the vaginal epithelium (without uterine growth) may be produced by vaginal administration of 1/200th of a rat unit of "Progynon" (kindly supplied by the Schering Corporation, New York).

¹ Pratt, J. P., and Smeltzer, M., *Endocrinol.*, 1929, **13**, 320.

² Powers, H. H., Varley, J. R., and Morrell, J. A., *Endocrinol.*, 1929, **13**, 395.

† We are indebted to the Nursing Department of the Providence Hospital, Oakland, California, for obtaining urine samples from 4 nulliparous nurses from 19 to 21 years of age. Their physical and dietary habits were as equal as hospital routine would allow. They had normal menstrual cycles varying from 25 to 29 days with the period of flow varying from 4-6 days. Cases 3 and 4 had acne vulgaris, otherwise no gross deviations from the normal were observed.

cycle, extracted immediately, or stored at 0°C. Half of the 24-hour sample was evaporated *in vacuo* over a steam bath to a salty sludge, (a few drops of capryl alcohol being added to lessen foaming). The sludge was extracted with two 100 cc. portions of hot absolute C_2H_5OH and the insoluble material discarded. The alcohol was distilled off, and the residue neutralized with N/1 or stronger NaOH. A graded series of dilutions of this extract was prepared with distilled water so that the 0.02 cc. used in testing represented original urinary volumes of from 0.05 to 1.0 cc. 0.01 cc. of each dilution was introduced twice (24-hour interval) into the vaginae of 3 full-grown rats ovariectomized at least 10 days previously.† The minimal effective urinary equivalent was considered the smallest amount causing cornification in the vaginae of at least 2 of the 3 rats, 24 hours after the second dose. After an interval of 5 days or more, the rats were used again.

TABLE I.
Vaginal Units of Estrin Excreted Daily by Women.

Case	Day of Cycle	24 hr. Urine cc.	Minimal Effective Urine Equivalent cc.	"Vaginal" Units per 24 hour	Units per litre
1	7	1300	0.8	1620	1250
"	14	600	0.4	1500	2500
"	21	1680	0.4	4200	2500
"	28	1700	0.4	4250	2500
2	7	1200	0.4	3000	2500
"	14	1000	0.1	10000	10000
"	21	960	0.2	4800	5000
"	28	930	0.6	1550	1666
3	7	950	0.4	2375	2500
"	14	1300	0.4	3250	2500
"	21	1160	0.4	2900	2500
"	28	1140	0.6	1900	1666
4	7	1920	0.4	4800	2500
"	14	1700	0.1	17000	10000
"	21	1930	0.2	9650	5000
"	28	2000	0.4	5000	5000

Table I contains the figures for the number of minimal effective doses of estrin per litre, as well as the daily estimates. They show that one may detect a rat vaginal-cornification unit in from 0.1 to

† A Breed and Brew milk pipette (U. S. Public Health Association Standard) calibrated to deliver 0.01 cc. milk was used. A preliminary swabbing of the vaginal mucosa with dry cotton allows for better retention of the extract. If the tip of the pipette is at the cervix, and if the pipette is rotated in the vagina during and after the administration, a more even distribution of the hormone is ensured, and the likelihood of reflux minimized.

0.8 cc. of a normal woman's urine. The 2 samples showing the highest unitage happened to have been taken in the mid-interval and therefore when a ripened or recently ruptured Graafian follicle may have been influential.

8462 C

Reactions of Mammary Glands of Normal and Hypophysectomized Male Guinea Pigs to Female Sex Hormone.

WILLIAM R. LYONS AND RICHARD I. PENCHARZ. (Introduced by Herbert M. Evans.)

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It has become increasingly important during these last few years of intensive experimentation on the hormonal control of the mammary apparatus to discriminate between the respective rôles played by the primary female sex hormone (estrin) and the anterior pituitary mammary-stimulating hormone (mammatropin), in inducing growth and secretory activity of the breast.

Several years ago in the course of experiments on the effects of purified gonadotropic hormone in hypophysectomized rats¹ it was noticed that although the female genital tract responded to the hormones of the stimulated ovaries, the mammary apparatus showed only a poorly-developed duct system. These findings pointed to a greater importance of the anterior pituitary as a source of a mammary growth-promoting factor than is admitted by some investigators. To further determine the degree of mammary stimulation possible through estrin, it was decided to use hypophysectomized male guinea pigs, since Laqueur and others² have shown that the intact male guinea pig responds to estrin treatment by mammary growth and lactation. Asdell and Seidenstein³ found the development of the mammary glands of ovariectomized, hypophysectomized rabbits injected daily for 15 days with 25 rat units of estrin plus 4 rabbit units of progestin almost the same as ovariectomized, non-hypophysectomized rabbits similarly treated. Apparently they did not study the effect of estrin alone.

¹ Evans, H. M., Pencharz, R. I., and Simpson, M. E. Unpublished observation.

² Laqueur, E., Borchardt, E., Dingemanse, E., and De Jongh, S. E., *Deut. med. Wchnschr.*, 1928, **54**, 465.

³ Asdell, S. A., and Seidenstein, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 931.

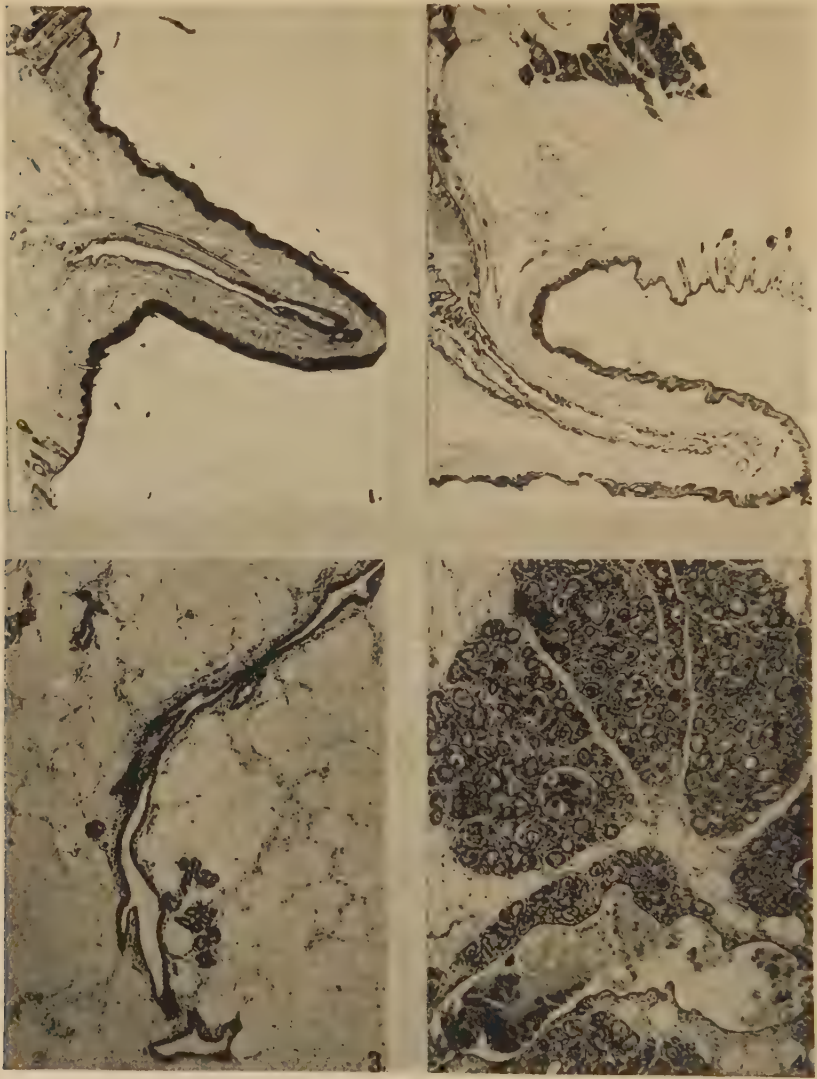


FIG. 1.

Section of nipple of hypophysectomized male guinea pig injected with estrin. $\times 10$.

FIG. 2.

Section of nipple of normal male guinea pig injected with estrin. Part of the alveolar system and ducts distended with milk are shown. $\times 10$.

FIG. 3.

Section of mammary gland of hypophysectomized male guinea pig injected with estrin. $\times 50$.

FIG. 4.

Section of mammary gland of normal male guinea pig injected with estrin. $\times 50$.

Our experimental material included 6 normal and 6 hypophysectomized male guinea pigs. The operation had been carried out by the parapharyngeal route, an approach which permits removal of all the pituitary tissue below the diaphragma sellae, but leaves the infundibular stalk with its accompanying pars tuberalis cells intact.

All of the animals were injected with Progynon* for one month. During this period each had received approximately 1800 rat units of female sex hormone.

The nipples of the hypophysectomized animals had grown from an original length of 2 mm. to 6 mm., those of the intact guinea pigs from 2 mm. to 8 mm. None had lactated as yet, but during the second month by injecting 200 rat units of estrin daily for 2 days, and then 2 rat units daily for the next 5 days as suggested by De Jongh and Dingemanse,⁴ it was possible to cause lactation in all of the intact animals within a month (2 lactating within a week). Four of the 6 hypophysectomized animals survived 2 months of injections and showed nipple development almost equal to that in the intact series, but one could express from their mammary glands only a few drops of a clear serous fluid such as may be obtained from the normal guinea pig at estrus. From the 6 intact animals milk could be readily expressed in small streams.

The accompanying photomicrographs illustrate histologically the main findings. Fig. 1 and 2, nipple sections of the hypophysectomized and intact estrin-injected males, respectively, show that this part of the male breast responds equally well in normal and hypophysectomized guinea pigs, attaining the dimensions of a normally-lactating female. Fig. 3 shows the limited growth of ducts and alveolar buds obtained in the hypophysectomized animals. Estrin promoted mitotic proliferation of solid outgrowths from the ducts with subsequent canalization. Fig. 4 shows a gland section of one of the injected intact males with functional alveolar development comparing favorably with that of normal lactation.

Since hypophysectomized male guinea pigs appear no less susceptible than normals to the effect of estrin as shown by nipple growth, and since this hormone does not cause in them lactation or growth approximating that of the intact animal similarly injected, it would seem that the presence of the pituitary is necessary for functional growth of the mammary gland. This is consistent with our former finding that the mammary glands of guinea pigs hypo-

* The progynon in oil kindly supplied to Dr. H. M. Evans by Schering Corporation contained 10,000 R.U. per cc.

⁴ De Jongh, S. E., Dingemanse, E., *Arch. Neerl. de Physiol.*, 1931, **16**, 288.

physectomized in pregnancy rapidly regress, especially following parturition.⁵

8463 C

The Dopa Reaction in Amphibia.

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From the School of Biology, Stanford University.

Considerable evidence has accumulated indicating that the dopa reaction is specific for distinguishing melanoblastic cells in mammals (Bloch¹). According to Bloch the dopa is oxidized to dopa-melanin by a specific oxidase which is present only in cells capable of forming melanin normally. The theory further assumes that dopa (3,4-dioxyphenylalanin) which is an intermediate oxidation product of tyrosine² is the normal precursor of melanin in animals. In mammals the reaction occurs only in pigmented regions of the epidermis, never in white areas. However, in adult amphibia dopa reacts in all regions of the epidermis whether pigment is present or not.³ The reaction is most intense in the outer layers of the epidermis in contrast to the conditions in mammals where the reaction is most intense in the basal cells (*stratum germinativum*).

In the present work the skin of 4 different urodeles was used with results which confirm those of Schmidt (Table I). Laidlaw's technic⁴ was used with the exception that frozen sections were not made. Small pieces of skin were fixed for 2 hours in 5% formalin, washed 10 minutes in distilled water and placed in 0.1% buffered dopa solution at a temperature of 36°C. for 6-11 hours. The controls were put in the phosphate buffer solution without dopa. The pieces of skin were then imbedded and sectioned in the usual way. Apparently, the dopa solution penetrates the epidermis rapidly, since the reaction is the same at the cut edges as elsewhere.

⁵ Pencharz, R. I., and Lyons, W. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1131.

* National Research Fellow in the Biological Sciences.

¹ Bloch, B., *Z. ges. exp. Med.*, 1917, **5**, 179; *Centralbl. Haut- und Geschlechts-Krankheit*, 1923, **8**, 1; also, summary by Biedermann, W., *Ergebn. d. Biol.*, 1928, **4**, 360.

² Raper, H. S., *Physiol. Rev.*, 1928, **8**, 245.

³ Schmidt, W. J., *Dermatol. Z.*, 1919, **27**, 284.

⁴ Laidlaw, G. F., *Anat. Rec.*, 1932, **53**, 477.

TABLE I.
The Dopa Reaction in Various Tissues of Amphibia.

		White Axolotl	Black Axolotl	<i>Triturus</i> <i>similans</i>	<i>Triturus</i> <i>pyrrhogaster</i>
1. Leydig cells	Occurrence	pres.	pres.	abs.	abs.
	Dopa reaction	—	—	—	—
2. Epidermal melanophores	Occurrence	abs.	"	"	"
	Dopa reaction		?		
3. Epidermal melanin granules	Occurrence	abs.	pres.	pres.	abs.
4. Epidermal cells	Dopa reaction	+	++	+++	++++
5. Mucous glands	Occurrence	abs.	abs.	pres.	pres.*
	Dopa reaction			+	+
6. Granular glands	Occurrence	abs.	abs.	pres.	pres.*
	Dopa reaction			—	—
7. Dermal melanophores	" "	—	±	?	?
8. Blood cells	" "	±	±	+	+

— = negative reaction.

+ = positive reaction (the number of plus signs in row 4 indicates the relative intensity of the reaction).

± = slight or questionable reaction.

? = no decision possible because of intense normal pigmentation (*cf.* dermal melanophores.)

*Some of the skin glands of this species are apparently compound, having both granular and mucous parts, while others are separate. Only the mucous portions give the reaction.

The strength of the reaction seems to be correlated with the total amount of pigment in the dermal melanophores. Thus,

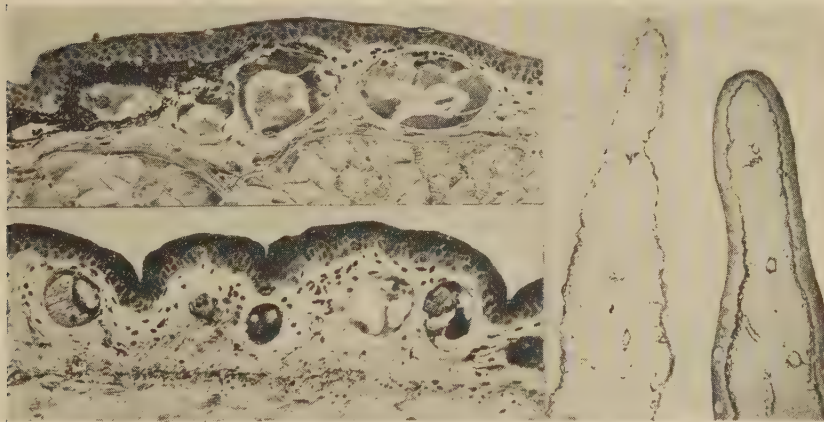


FIG. 1.

Sections of ventro-lateral skin of an adult *Triturus pyrrhogaster*. Above, control which shows dermal melanophores on the left. The epidermis contains no melanin. Below, section of skin which was placed in dopa solution for 11 hours. Heavy deposit of dopa-melanin in outer layers of epidermis and some skin glands (especially gland in center). Photos, $\times 75$. Heidenhain's haematoxylin and eosin.

FIG. 2

Unstained sections through the dorsal fin of an adult black axolotl. Left, control; right, dopa-treated for 11 hours. The outer layers of the epidermis show a moderate reaction, except for the Leydig cells (circular colorless areas). The dermal melanophores seem to have shown a slight reaction. Photo, $\times 28$.

the reaction increases in intensity in the epidermis of the 4 species in the following order (Table I, row 4): Albino axolotl, black axolotl (Fig. 2); *Triturus similans*;⁵ *T. pyrrhogaster* (Fig. 1). While this general correlation seems to indicate that the reaction has some significance in regard to pigment formation, other results do not harmonize with this. The reaction takes place quite as well on the ventral sides where no dermal melanophores occur (*pyrrhogaster*, *similans*) as it does where melanophores are numerous. In addition, there is no relation between the presence or absence of epidermal melanin granules and the dopa reaction (compare rows 3 and 4). Thus, dorsal epidermis of *T. similans* contains melanin granules; ventral epidermis does not. Nevertheless, the reaction proceeds with the same intensity in both regions.

These results, while not in agreement with those in mammals, do not necessarily exclude the possibility that dopa may play some part in normal melanin formation in amphibia. For example, if it could be shown that the dark regions of the skin produced both the oxidase and the chromogen (dopa) and that the light skin produced the oxidase, as it apparently does, but no chromogen, then these experiments would fall into line with those on mammals.

Evidence from embryonic transplantations indicates that the epidermis is active in inducing melanin formation in prospective dermal melanophores.⁶ Thus, it is not improbable that the epidermis of amphibia, even though not strictly melanoblastic, plays an important rôle in the enzyme-chromogen system responsible for melanin formation.

Further difficulties of interpretation are introduced by the fact that a number of other tissues which presumably have nothing to do with pigment formation give positive reactions. Thus, red blood cells (row 8), mucous skin glands (row 5) and the secretions of the unicellular glands of the intestine and rectum (*similans*) are dopa-positive. Preliminary experiments with embryos of *T. pyrrhogaster* show that not only the neural crest cells (prospective melanophores) and epidermis, but also some of the primitive blood cells in the mid-ventral blood-island and an occasional yolk cell are dopa-positive. As in adults, the reaction occurs in those cells which are of some importance in melanin formation as well as in other cells which have no such significance. This result is in contrast to those reported in abstract by Blanchard and McCurdy,⁷ who

⁵ New species. Twitty, V. C., *Copeia*, 1935, **2**, 73.

⁶ DuShane, G. P., *Science*, 1934, **80**, 620; *J. Exp. Zool.*, 1935, **72**, 1.

⁷ Blanchard, E. W., and McCurdy, H. M., *Anat. Rec.*, 1934, **60**, (Suppl.) 62.

found that only the ectoderm and neural crest were dopa-positive in *Triturus torosus* embryos.

No definite answer can be given to the question of the significance of the dopa reaction in amphibian melanin formation. The reaction is not "absolutely specific" for melanoblasts in this group as Bloch¹ has claimed for mammals, but it is possible that the reactions of non-melanoblastic cells are adventitious.

The evidence in this paper is sufficient to show that a cell which gives a positive dopa reaction is not necessarily melanoblastic. Consequently, the dopa reaction is not an adequate criterion for distinguishing melanoblasts or prospective melanophores in amphibia.

8464 P

Effects of Various Diets, Cholesterol and Choline on Liver Lipids of the Rat.

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It has long been known that the diets high in fat cause a marked increase in the neutral fat of the liver. Best and his coworkers^{1, 2} have shown that this can be prevented or cured in the rat by administration of choline. Moderately fatty livers were produced by these workers when they fed rats exclusively on sucrose or on any other choline-free diets. Cholesterol in doses of 100 mg. daily had a similar effect. This change was found to be preventable, however, by the administration of as little as 5 mg. of choline daily. It has been shown by Schonheimer and Yuasa,³ and confirmed by Page and Menschick,⁴ Okey,⁵ Chanutin and Ludewig,⁶ Sperry and Stoyanoff,⁷ and others, that cholesterol feeding causes a marked increase in the cholesterol of the liver. Chanutin and Ludewig found that added carbohydrate accelerated while fat inhibited the deposition of cholesterol in the liver of cholesterol-fed rats. Little attention appears to have been given to the phospholipid fraction in the liver

¹ Best, C. H., and Ridout, J. H., *Am. J. Physiol.*, 1933, **105**, 6.

² Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1935, **83**, 255.

³ Schonheimer, R., and Yuasa, D., *Z. f. Physiol. Chem.*, 1929, **180**, 5.

⁴ Page, I. H., and Menschick, W., *J. Biol. Chem.*, 1932, **97**, 359.

⁵ Okey, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1003.

⁶ Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1933, **102**, 57.

⁷ Sperry, W. M., and Stoyanoff, V. A., *J. Nutrition*, 1935, **9**, 131.

under the various conditions mentioned, except for the finding by Chanutin and Ludewig that lecithin remained normal after cholesterol feeding. The specific effects of high-protein diets in this connection have not been recorded.

The results presented here supplement previous studies by supplying data on these points. The analyses reported were carried out on livers from rats used in a previously reported series of experiments on the influence of diet and other factors on the lipid content of the brain.⁸ Four rats from our own stock colonies were placed at the age of 3 weeks on each of the following special regimens: 1. standard control diet (Chanutin and Ludewig), 2. high-fat (85%), 3. high-carbohydrate (80%), 4. high-protein (80%), 5. Chanutin diet plus 5.0% added cholesterol, 6. Chanutin diet plus 2.5% cholesterol, 7. the latter plus 1.3% choline. The diets were made up from casein, lard, starch, cod liver oil, yeast powder, water and Osborne-Mendel salt mixture. The experiments extended over periods varying between 44 and 105 days. For each group of animals on special diet a control group was kept on a standard mixed diet for the same period of time. At the end of the experimental period the animals were killed by sudden decapitation, after which their livers were promptly removed and weighed. An aliquot portion of each organ was taken for determination of the water content. The remainder was immediately prepared for extraction by the procedure of Osato and Heki.⁹ The total fatty acids, cholesterol and phospholipids were then determined by the methods of Bloor.^{10, 11}

The results may be summarized briefly. Expressed in percentage of dry weight, the livers of the control rats fed on the standard diet for 105 days were found to contain on the average, total fatty acids, 14.34, cholesterol 1.03 and phospholipids 12.37. In comparison with these values those for the animals on special diets for the same length of time were as follows: *high-fat diet*, total fatty acids 33.05, cholesterol 1.45 and phospholipids 16.72%; *high-carbohydrate diet*, total fatty acids 24.32, cholesterol 1.25 and phospholipids 13.56%; *high-protein diet*, total fatty acids, 15.69, cholesterol 1.16 and phospholipids 33.92%.

Livers of animals taking the Chanutin control diet plus 5.0%

⁸ Stoesser, A. V., Petri, K. A., and McQuarrie, Irvine, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **82**, 761.

⁹ Osato, S., and Heki, M., *J. Biol. Chem.*, 1930, **87**, 543.

¹⁰ Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

¹¹ Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

cholesterol contained on the average no more of any of the lipids determined than those on the diet plus 2.5% cholesterol. However, all lipid fractions were higher in the livers of rats which were on the diet plus cholesterol 96 days than in those of rats which were killed on the 44th day. Values for the control animals for the two periods did not vary significantly. Contrary to the finding of Chanutin and Ludewig, the phospholipid content of the liver in our rats was found to be increased by approximately 50% as a result of prolonged cholesterol feeding. Choline effectively prevented any significant increase in the neutral fat and phospholipid content of the liver from cholesterol feeding but only partially inhibited the extra deposition of cholesterol.

New York Section

New York Academy of Medicine, January 15, 1936.

8465 P

Detoxication of Phenylacetic Acid by the Chimpanzee.

FRANCIS W. POWER. (Introduced by Leopold R. Cerecedo.)

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The chemical defense mechanism of the lower animals, including the monkey,¹ with respect to phenylacetic acid results in a conjugation with glycine; that of man, however, results in the formation of the glutamine conjugate.

Some time ago an opportunity was afforded me at the New York Zoological Park of ascertaining what this detoxication mechanism might be in the anthropoid ape. A young chimpanzee ("Buddy"), at that time about 2½ years old and weighing about 10 kilos was placed in a metabolism cage and two 24-hour samples of urine collected. Then four 1.5 gm. portions of phenylacetic acid (mixed with bone meal) were fed to the animal at successive 12-hour intervals and the urine collected 12 hours after each feeding. He received his regular diet of milk, bananas and eggs during the whole experiment.

The bulk of the urine was evaporated to dryness, the residue made acid to congo red with H₂SO₄, mixed with dry sand and extracted exhaustively in a Soxhlet apparatus with absolute ethyl acetate. The crystals which appeared were freed from urea and purified² and were found to possess all the properties of phenylacetylglutamine. The melting point was the same, 106°-109°; a 50-50 mixture of the pure substance and the crystals from this experiment melted at 102°-105°; the total nitrogen (Kjeldahl) was 10.05% as against 10.61% calculated; and on alkali hydrolysis 96.4% theoretical ammonia was evolved as compared with a 98.9% recovery from the pure substance run in the same apparatus.

¹ Sherwin, C. P., *J. Biol. Chem.*, 1917, **31**, 307.

² Hawk, P. B., *Practical Physiological Chemistry* (1926), 9th edition, p. 304.

The fact that all these figures run a little low suggests a slight admixture with phenaceturic acid, but I did not attempt an exact estimation of its proportion, if any.

The extent of the detoxication was determined as follows: duplicate 50 cc. portions of the urine were made about 6 normal with respect to H_2SO_4 , boiled 2 hours under a long spiral condenser, the latter washed down into the flask with a little water and the mixture shaken out with one 50 cc. and three 25 cc. portions of chloroform. The combined chloroform extracts were filtered through cotton and titrated with 0.0718 normal sodium ethylate (metallic sodium dissolved in absolute alcohol) using phenolphthalein as indicator. I have found this process to be adequate for complete hydrolysis of phenylacetylglutamine in urine, a recovery of 99.5% being obtained as an average of 6 determinations on human urine after making allowance for the titratable acidity arising from the urine itself after putting it through the identical process. The recovery of phenylacetic acid as determined by this method is shown in Table I.

TABLE I.

12-hour Urine Samples	Volume	Phenylacetic acid recovered from the respective 12-hour samples
	cc.	gm.
After 1st feeding	880	1.03
" 2nd "	760	1.19
" 3rd "	790	1.31
" 4th "	440	1.47

Thus 5 out of 6 gm. fed were recovered. The above figures have been corrected for the titratable acidity of the chloroform extract of the control urines which were hydrolyzed in the same way; this blank amounted to 0.12 gm. per 12-hour period, calculated to phenylacetic acid. Reduction tests were made on all the urines by Somogyi's method; calculated to glycuronic acid; the reduction corresponded to 0.16 gm. per 12-hour control period, but only after the last of the four 1.5 gm. doses did the reducing power of the urine exceed that of the controls, and then only by an insignificant amount.

The incomplete recovery may best be ascribed to a retention of phenylacetic acid; this has been observed before in human subjects fed rather large doses.³ This chimpanzee was receiving about 160 mg. per kilo of body weight every 12 hours, which is about twice as much as would be considered a fairly heavy dose for a human subject. The animal showed no untoward symptoms, however,

³ Ambrose, A. M., Power, F. W., and Sherwin, J. *Biol. Chem.*, 1933, **101**, 669.

and the urine was always negative for albumen. The 24-hour urine volumes of the control period averaged only 590 cc., as compared with 1435 cc. for the experimental period, an effect which we notice with human subjects also.

Owing to the obvious impossibility of proving descent from similarity I do not propose these data in relation to any theory on human descent; however, I do think that they reveal an interesting point in intermediary metabolism which I hope to investigate further.

I wish to acknowledge with gratitude the very generous co-operation of the authorities at the Bronx Zoo, especially that of Dr. Charles V. Noback and his assistant.

8466 C

Effect of Iodine on Absorption of Cholesterol.*

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It is now an established and unequivocal fact that cholesterol is readily absorbed from the gastro-intestinal tract and that an increase may be demonstrated in the thoracic duct chyle after feeding. Relatively little is known, however, about the factors influencing this absorption. Thus, when a rise in the blood cholesterol of rabbits was prevented for several months by feeding potassium iodide with the cholesterol,¹ it was considered possible that the iodide in some way prevented the absorption of the sterol, but no information bearing on this point was available.

It seemed possible to approach the problem by feeding cholesterol to normal and iodized animals and determining the level of the cholesterol in the chyle. Unfortunately the small size of the thoracic duct in rabbits made it infeasible to use them for this purpose although it would have been desirable to carry out the work on the same species of animal used in the previous experiments. Dogs seemed to offer the least technical difficulty and accordingly were used with the realization that, while results obtained in omnivores could not be applied directly to herbivorous animals such as rab-

*Aided by a grant from the Josiah Macy, Jr., Foundation.

¹ Turner, K. B., and Khayat, G. B., *J. Exp. Med.*, 1933, **58**, 127.

bits, nevertheless it might be possible to answer the question as to whether the iodide influenced the absorption of the sterol.

The dogs chosen for experimental subjects were long-necked hounds about one year old, weighing 9 to 13 kg. Pentobarbital sodium (Nembutal, Abbott) on the average of 40 mg. per kg. body weight given intraperitoneally induced deep anesthesia for about one hour. This was usually sufficient for the operative procedure. After obliterating all visible lymphatics on the right side of the neck which might possibly transport chyle, as suggested by Drinker and Field,² a thoracic duct fistula was made on the left side. With minor variations the method used was one described by Krupping and Kummell.³ The chyle was shunted into an isolated segment of the left external jugular vein, into which a paraffined glass cannula had been tied. In common with other investigators, we experienced difficulty in collecting chyle for more than 6 or 8 hours. The collection of chyle was stopped when delivery from the cannula became slow or intermittent, or if the resulting specimen became thin or contaminated with blood. The animal was allowed to recover at the end of the operation, but was usually still dazed or sleeping when the flow of chyle ceased.

The chyle was allowed to clot in the collecting bottles. The clot was then ground up thoroughly and the cholesterol determined as in blood by the method of Bloor, Pelkan and Allen.⁴

As a control group, 9 dogs were used. These animals were fasting excepting for the fact that they were given 250 cc. of 40% cream at a variable time before operation in order to assure visualization of the thoracic duct. The seemingly long interval that elapsed between the feeding of the cream and the collection of specimens was intentional as it was desirable to have the process of absorption well under way before subjecting the animal to the anesthetic and the trauma attendant upon the operative procedures. After operation when the flow of chyle was established 2-hour samples were collected as long as the flow remained free and profuse. Sixteen samples from the 9 animals were secured. The results are given in Table I. The cholesterol content of the chyle varied from 69 to 152 mg. per 100 cc. with an average of 121 mg.

In a second group were 6 dogs. These were given cream as in the control group except for the fact that 10 gm. of cholesterol were

² Drinker, C. K., and Field, M. E., *Lymphatics, Lymph and Tissue Fluid*, Williams and Wilkins Company, 1933, 175.

³ Krupping, H. W., and Kummell, H., Jr., *Beitr. z. Klin. Chir.*, 1924, **132**, 123.

⁴ Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

TABLE I.
Chyle Cholesterol of Dogs Fed Cream.

Dog No.	Hr. after feeding	Chyle cholesterol mg./100 cc.
151	6-8	128
162	4-6	69
163	4-6	131
185	2-4	152
214	7-9	114
	9-11	105
	11-13	136
303	14-16	116
	16-18	127
304	7-9	136
	9-11	134
	11-13	136
420	13-15	120
	15-17	112
	17-19	88
424	12-13	127
Aver.		121

mixed with the cream. There was one exception to this procedure. One dog (No. 185) was given cream alone and a fistula produced. After a sample of chyle had been taken (Table I), cream and cholesterol were given by stomach tube and additional specimens obtained for analysis. A total of 12 samples was secured from the 6 dogs after the cholesterol feeding. The results are given in Table II. The range of the chyle cholesterol was from 108 to 258 mg. with an average of 169 mg.

TABLE II.
Chyle Cholesterol of Normal Dogs Fed Cream and Cholesterol.

Dog No.	Hr. after feeding	Chyle cholesterol mg./100 cc.
176	6-8	180
	8-10	180
185	0-2	142
	2-4	162
	4-5	175
224	3-5	114
	5-7	108
	7-8	142
285	15-17	208
421	13-15	174
422	13-15	258
	15-17	182
Aver.		169

In the final group were 4 dogs. For one week before operation each animal was given daily 1 cc. of a saturated solution of potassium iodide by mouth. Cream with an added 10 gm. of cholesterol

was given preoperatively as in the preceding group. Ten specimens were secured in the usual manner from the 4 dogs. The results are given in Table III. The chyle cholesterol varied from 164 to 221 mg., with an average of 193 mg.

TABLE III.
Chyle Cholesterol of Iodized Dogs Fed Cream and Cholesterol.

Dog No.	Hr. after feeding	Chyle cholesterol mg./100 cc.
323	15-17	213
	17-19	171
370	13-15	170
	15-17	164
	17-19	180
371	13-15	208
406	11-13	221
	13-15	190
	15-17	208
	17-19	206
Aver.		193

From the foregoing it is evident that cholesterol absorption from the gastro-intestinal tract is not prevented by the previous administration of potassium iodide. The average chyle cholesterol for the dogs receiving cream alone was 121 mg. compared to 169 mg. for the normal animals fed cholesterol and cream, and 193 mg. for the iodized group. The results are further summarized in Fig. 1. The figures given perhaps suggest that the iodide may even augment

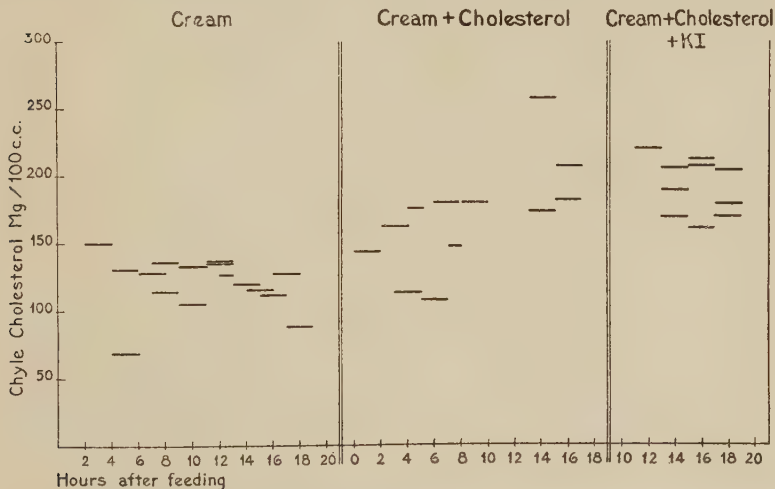


FIG. 1.
Chyle cholesterol values for the 3 groups of dogs. Each horizontal line represents one determination.

the absorption of cholesterol. However, when only the specimens obtained 11 hours or more after the feedings are considered this apparent discrepancy disappears. For the control group the average chyle cholesterol then becomes 120 mg.; for the normal dogs given cream and cholesterol, 206 mg.; and for the iodized animals, 193 mg.

Conclusion. Potassium iodide does not prevent the absorption of cholesterol from the gastro-intestinal tract of dogs.

8467 P

Vaccination of Human Subjects with Virus of Human Influenza.

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The immunization of ferrets and mice^{1, 2} with human influenza virus has been reported. When injected into these animals by the subcutaneous or the intraperitoneal routes the virus elicits no evidence of infection, but the animals so treated develop circulating antibodies against the virus and become actively resistant to the infectious agent introduced by the intranasal route. More recently it has been possible to demonstrate the same effects with virus grown outside the animal body in tissue culture medium.^{3, 4}

The successful cultivation of the virus in artificial medium immediately eliminated many of the undesirable features, such as the possibility of bacterial contaminants, extraneous viruses, etc., which constantly attend the use of virus from infected animals for studies in human individuals. After preliminary tests upon 3 human subjects which demonstrated that the artificially cultivated virus injected subcutaneously produced no serious reactions, a group of adult human volunteers was selected to determine the immunizing capacity of culture virus.

From among the subjects available, those individuals were selected whose serum beforehand exhibited the least protective power against the human influenza virus, as measured by its capacity to protect white mice against a high concentration (10% suspension)

¹ Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1935, **62**, 505.

² Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Brit. J. Exp. Path.*, 1935, **16**, 291.

³ Francis, T., Jr., and Magill, T. P., *Science*, 1935, **82**, 353.

⁴ Magill, T. P., and Francis, T., Jr., *J. Exp. Med.*, in press.

of mouse passage virus. From each individual a sample of serum was obtained immediately before the first injection of virus and again before each subsequent inoculation. To each of 10 individuals 3 doses of culture virus, 0.5 cc., 1.0 cc., and 1.0 cc., respectively, were given subcutaneously at weekly intervals. After 2 to 3 weeks another dose of 2.0 cc. of culture virus was given to each individual by the same route, and approximately 10 days later the final sample of serum was obtained. In one group of 4 individuals, doses of 1.0 cc., 1.0 cc., and 2.0 cc., respectively, were given at weekly intervals.

The only reactions observed were mild local erythematous areas, sometimes slightly tender, at the site of injection. The erythema was usually first apparent in 18 to 24 hours after inoculation, and subsided in another 24 hours. No constitutional or significant febrile reactions, no respiratory symptoms or evidence of infection were noted.

The sera collected at intervals in the course of vaccination were then tested by mouse protection tests for their capacity to neutralize a 10% suspension of mouse passage virus. All tests of the serum of a single individual were made at one time; in fact, the sera of 4 or more individuals were always included in the same experiment. Control tests were run simultaneously with a serum which was known to protect mice completely and with another serum which was known to have no protective action. The development of circulating antibodies against the virus was then determined by comparing the neutralizing capacity of the serum after vaccination with that of the serum before the injections were begun. Furthermore, the titer of protective substances in the sera of different individuals could be compared by relating the results to those obtained with the control sera.

Table I presents the results of tests made with the serum of each subject taken before vaccination was begun and after the final inoculation. The results show that while the antibody response varied among the different individuals, in all, except possibly subject H. S., a sharp increase in the protective capacity of the serum occurred. In 4 instances the serum before vaccination exhibited no neutralizing action against the virus, in the dosage employed, while subsequent to the course of inoculations, the serum of the same individuals exerted essentially complete neutralization. In those subjects whose sera afforded partial protection to mice before vaccination, an increased protective capacity of the serum after vaccination was also noted.

The questions of persistence of antibodies following vaccination

TABLE I.
The Development of Antibodies in Human Subjects Following Vaccination with Human Influenza Virus in 1935.

Subject	Serum No.	Date	Date of Test	Tested Against PR8 Strain Mouse Passage Virus			
				Severity of Pulmonary Lesions in Mouse			
				No. 1	No. 2	No. 3	No. 4
L.A.	1.	10/19	12/12	++++*	++++*	++++*	++++*
	6.	12/4	"	+	0	±	0
D.T.	1.	10/19	"	++++*	++++*	++++*	+++
	6.	12/4	"	0	0	0	+
W.M.	1.	10/26	"	+++	+++	++	+++
	5.	12/4	"	0	0	+	0
A.T.	1.	11/2	"	+++	+++	+++	±
	5.	12/11	"	0	0	0	0
F.R.	1.	10/26	"	+++	+++	++	+++
	5.	12/4	"	0	0	0	0
S.O.	1.	10/19	"	++	+++	+++	+++
	6.	12/4	"	+	0	+	0
M.R.	1.	10/26	"	++	++	+++	++
	5.	12/4	"	0	±	0	+
W.H.	1.	10/19	"	+	++	+	+
	6.	12/4	"	0	0	0	0
H.S.	1.	10/26	"	++	0	+	+
	5.	12/4	"	0	0	+	0
T.F.	1.	9/16	11/14	++++*	++++*	++++*	—
	4.	11/7	11/14	0	0	0	—
E.Me.	1.	10/31	12/4	++++*	++++*	++++*	++++
	4.	11/23	"	0	0	0	+
D.D.	1.	10/31	"	++++*	+++	+++	++
	4.	11/23	"	0	0	0	0
E.M.	1.	10/31	"	++	++	++	+++
	3.	11/14	"	0	0	0	0
F.F.	1.	10/31	"	++++*	++++*	++++*	+++*
	4.	11/23	"	++	+	++	++
R.D.	Neg. Control	"	"	++++*	++++*	++++*	++++*
		12/12	"	++++*	++++*	++++*	++++*
SS.	Pos. Control	12/4	"	0	0	0	0
		12/12	"	0	0	+	0

O = No pulmonary lesions.

± to ++++ = Degree of pulmonary involvement.

* = Mouse died.

with the living virus and the significance of these antibodies in relation to immunity to the natural disease must await further study for their solution. Nevertheless, the present results show clearly that the subcutaneous inoculation of artificially cultivated, active human influenza virus into human individuals elicits the production of specific protective substances, without causing any unfavorable reactions in the recipient.

8468 C

Tissue Cultures as a More Sensitive Method Than Animal Inoculation for Detecting Equine Encephalomyelitis Virus.

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Cultures consisting of minced chick embryo tissue suspended in Tyrode's solution¹ have been found favorable for the growth of equine encephalomyelitis virus. If it can be shown that cultures are equally or even more effective for revealing minimal quantities of virus in a material, or that cultures can bring to light the presence of virus when animal inoculation fails, a useful method will be available. Equally important is the fact that in a single test inoculum, a larger amount can be introduced into a culture than in an animal, since by intracerebral inoculation* a guinea pig can receive with safety a limiting quantity of only 0.2 cc., and a mouse 0.05 cc.

A comparison was therefore made of the relative sensitivity of cultures and of intracerebral inoculation of mice and guinea pigs for detection of the virus.

In Experiment 1, shown in Table I, a single disc Seitz filtrate of a 10% broth suspension of infected mouse brain tissue was diluted decimally to 10^{-8} dilution. Each of 4 mice received an intracerebral injection of 0.05 cc. from each dilution and a like number of cultures were inoculated with the same quantity. After 72 hours' incubation at 37°C. each culture was tested for the presence of active virus by intracerebral injection of mice with 0.05 cc. of the centrifuged supernatant fluid. Active virus was detected up through 10^{-6} dilution of the Seitz filtrate by animal test, and in 10^{-6} dilution by inoculation of tissue cultures.

In Experiments 1 and 3 (Table I) the cultures revealed virus in dilutions tenfold greater than the limiting dilutions active by direct intracerebral injection of animals.

Both "collar flasks"² and 50 cc. Erlenmeyer flasks have been employed as culture containers with equally good results. In large, flat bottomed flasks correspondingly greater quantities of culture suspension and of inocula can be used.

¹ Cox, H. R., Syverton, J. T., and Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 896.

* All such operations were done with the aid of deep ether anesthesia.

² Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

TABLE I.

Experiment	Substance and final dilution of virus tissue	Quantity inoculated in each culture or animal	Results of inoculation			
			Tissue culture	Animal†		
				Guinea pig	Mouse	
		cc.				
1	Filtrate M.B.	10 ⁻⁴	.05	4/4‡	—	4/4
		10 ⁻⁵	.05	4/4	—	4/4
		10 ⁻⁶	.05	2/4	—	0/4
2	Filtrate G.P.B.	10 ⁻³	.1	3/3	3/3	—
		10 ⁻⁴	.1	3/3	3/3	—
		10 ⁻⁵	.1	2/3	1/3	—
3	Filtrate G.P.B.	10 ⁻⁴	.1	3/3	3/3	—
		10 ⁻⁵	.1	3/3	3/3	—
		10 ⁻⁶	.1	2/3	0/3	—
4*	Supernatant M.B.	10 ⁻⁶	.05	5/5	—	5/5
		10 ⁻⁷	.05	5/5	—	5/5
		10 ⁻⁸	.05	5/5	—	2/5
5*	Supernatant M.B.	10 ⁻⁶	.05	4/4	—	4/4
		10 ⁻⁷	.05	4/4	—	3/4
		10 ⁻⁸	.05	2/4	—	1/4

M.B. = mouse brain; G.P.B. = guinea pig brain. For mice virus dilutions were prepared in hormone broth of pH 7.6; for guinea pigs in Tyrode's solution.

*Here dilutions were prepared from supernatant fluid obtained by spinning in an angle centrifuge at 4,000 r.p.m. for 30 minutes.

‡The denominator shows the number of cultures inoculated; the numerator the number of cultures showing virus.

†The denominator designates the number of animals receiving same substance as cultures and the numerator the number of animals dead from encephalitis.

Cultures of amounts of virus too small to be detected by direct animal inoculation contained, after incubation, virus in a concentration of at least 1:10,000. The virus present in such cultures had multiplied at least 700,000 fold for when 0.05 cc. of the test material was added to 3.5 cc. of medium, there was an immediate dilution of 1:70. After incubation the culture could be further diluted to at least 10,000 times and 0.05 cc. of the dilution induced lethal encephalitis in mice. Such increases in virus content were demonstrated in all of three cultures titrated.

To conclude, cultures of minced chick embryo tissue suspended in Tyrode's solution can reveal the presence of equine encephalomyelitis virus (Eastern strain) in dilutions which are inactive after intracerebral inoculation of mice and guinea pigs; the virus multiplies rapidly in cultures within 72 hours and, with more medium, a single inoculum larger than that employed in the animals can be tested for virus content.

The culture method has been applied in attempts to detect small amounts of active encephalomyelitis virus in formalized vaccines

which were inactive by animal inoculation. Moreover, multiplication of minute quantities of virus in tissue cultures was not inhibited by the presence in them of formalized vaccines in which the formalin was neutralized by ammonia. It was thus possible to determine that an amount of vaccine (1 cc.)—the immunizing dose—contained no detectable active virus.

8469 P

Host Age and Cell Metabolism in Mouse Lymphatic Leukemia.

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During the course of experiments on the relationship of cell virulence to cell metabolism, certain results indicated that the age of the host influenced the metabolism of transmitted leukemia cells. To investigate the effect of age, the following experiments were performed: Mice of 2 age groups (6-8 weeks and 6-8 months) were inoculated with the same transfer cells of a transmission line M-liver. Metabolic measurements, namely Q_{O_2} , $Q_{CO_2}^{O_2}$ and $Q_{CO_2}^{N_2}$ in Ringer's solution⁶ were made on the lymph nodes 4 days after inoculation. The mice were of strain C 58, 100% of which develop lymphatic leukemia after inoculation with line M-liver; 90% develop spontaneous lymphatic leukemia after 6 months of age.¹ So far as could be determined, *i. e.*, from palpation and blood counts before inoculation and autopsy, no evidence of spontaneous leukemia was found in mice used. A summary of the metabolic results is presented in Table I. The metabolism was significantly decreased in every characteristic studied. The decrease in Q_{O_2} of the leukemic lymph nodes parallels the decrease found in normal lymphoid tissue previously reported.² On the other hand the decrease in $Q_{CO_2}^{O_2}$ and $Q_{CO_2}^{N_2}$ is actually greater than the table indicates, for it has been found that the normal glycolytic rates are higher in 6-8-month-old mice than in 6-8-weeks-old mice. In other words the actual excess glycolysis, both aerobic and anaerobic produced by the leukemia cells is less than the values in the table.

⁶ Warburg, O., *Metabolism of Tumours*, London, Constable & Co., Ltd., 1930.

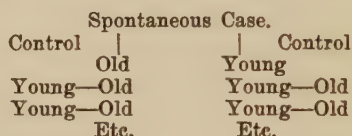
¹ MacDowell, E. C., and Richter, M. N., *Arch. Path.*, 1935, **20**, 709.

² Victor, J., and Potter, J. S., *Brit. J. Exp. Path.*, 1935, **16**, 243.

TABLE I.
Metabolic Effects of Age of Host on Cells of Line M Liver.
Young 6-8 weeks, Old 6-8 months.

	No. of Observation	Q _{O₂}		Q _{CO₂} ^{O₂}		Q _{CO₂} ^{N₂}	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
(1) Young	9	5.26±.12	.55	8.44±.25	1.11	18.36±.44	1.96
(2) Old	11	4.63±.10	.50	6.86±.22	1.09	16.39±.37	1.84
Difference		.63±.15		1.58±.33		1.97±.58	
(1) — (2)							
Diff.		4.2		4.8		3.4	
P.E. Diff.							

To test the reversibility of the age effect, the following experiments were performed. The metabolism of the lymphoid cells of a mouse of Strain C 58 with spontaneous lymphatic leukemia was determined. In addition to the other characteristics the R.Q. was measured in the presence of 0.2% glucose.³ The cells of the spleen were inoculated into old (6-8 months) and young (6-8 weeks) mice of this Strain C 58; then splenic transmissions were performed in 2 series through old mice with young controls and through young with old controls. Schematically this is represented as follows:



This line was called line O.

It seemed that in addition to age effects the experiment might throw some light on metabolism associated with changes in virulence. It has been found that the virulence of a transmission line of leukemia increases very rapidly in the early transfers. Thirteen transfers were studied. About 380 observations were made. The results are summarized in Table II. Table III shows a comparison of the

TABLE II.
Metabolic Rates of Lymph Nodes of Mice Inoculated with Line O.
Old 6-8 months, Young 6-8 weeks.

Group	Q _{O₂}		R.Q.		Q _{CO₂} ^{O₂}		Q _{CO₂} ^{N₂}	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
(1) Old-Old	4.65±.06	.48	.773±.003	.022	2.18±.06	.47	8.06±.25	1.98
(2) Old-Young	4.95±.08	.51	.840±.005	.027	2.65±.05	.33	9.33±.31	2.19
(3) Young-Young	5.31±.11	.75	.898±.003	.021	2.64±.16	1.05	9.70±.24	1.58
(4) Young-Old	4.81±.11	.68	.812±.004	.021	2.43±.15	.96	8.46±.33	2.09

³ Victor, J., and Potter, J. S., *Brit. J. Exp. Path.*, 1935, **16**, 253.

metabolic differences between the 2 age groups and the various methods of transmission. The statistically significant differences are italicized.

TABLE III.
Metabolic Differences of Lymph Nodes of Old and Young Mice Inoculated with Line O.

Comparison of	Q _{O₂}		R.Q.		Q _{O₂} CO ₂		Q _{N₂} CO ₂	
	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.	
		PE Diff.		PE Diff.		PE Diff.		PE Diff.
(2) — (1)	.30±.10	3.0	.067±.006	11.2	.47±.08	5.8	1.27±.40	3.2
(3) — (4)	.50±.15	3.3	.086±.005	17.2	.21±.22	1.0	1.24±.41	3.0
(1) — (4)	.16±.13	1.2	.039±.005	7.8	.25±.16	1.5	.40±.41	1.0
(2) — (3)	.41±.13	3.2	.058±.006	9.6	.01±.17	0	.43±.39	1.1

Two major conclusions may be drawn from these summaries. The first is that age has an effect upon the metabolism of transmitted leukemia cells. In older mice the R.Q. in the presence of glucose, Q_{O_2} , $Q_{CO_2}^{O_2}$, and $Q_{CO_2}^{N_2}$ are lower than in younger mice. If cells are transmitted through old mice and into younger the metabolism is higher with younger and *vice versa*. The effect of age is reversible. This reversibility of leukemia cell metabolism produced by host age is similar to that resulting from cell transmission through genetically different hosts.⁴ The second point is that passage through the young and old mice (the center portion of the scheme) was associated with the development of 2 parallel lines that were distinctly different metabolically. This has been observed before⁵ and is not necessarily correlated with passage through different age groups. Host age does not modify the inherent constitution of the leukemia cells but acts as a determining environmental factor on their metabolism.

⁴ Victor, J., and Potter, J. S., *J. Exp. Med.*, 1934, **60**, 547.

⁵ Victor, J., and Wintersteiner, M. R., *Am. J. Cancer*, 1934, **22**, 561.

Hormonal Factors Affecting Vaginal Smears in Castrates and After the Menopause.

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Papanicolaou and Shorr¹ have recently described the cytological characteristics of the vaginal smear found in castrates and in women after the menopause. As described by these authors, the smears contain "many leucocytes and a predominance of either non-cornified squamous cells with larger nuclei or of compact cells derived from the deeper layers of the vaginal epithelium, with large well-preserved nuclei."¹ By the administration of estrogenic substance hypodermically these investigators were able, in 14 out of 15 cases, to demonstrate a change in these smears so that they resembled the "follicular (copulative) phase in the normal female."¹ This phase is characterized by "leukopenia and epithelial cells of the squamous type, largely cornified with small pyknotic nuclei."² These effects on the smears disappeared several days after cessation of the treatment.

We have recently reported the finding of large quantities of luteinizing factor as well as follicle stimulating factor in both the urine and blood of castrates and natural menopause cases³ and found that both these factors disappear from the urine and blood following the administration of concentrated estrogenic substance (Progynon-B).⁴

The present investigation was undertaken to determine whether any relationship existed between the type of vaginal smear, the gonadotropic hormone excretion and the symptoms in post-menopause cases and in surgical and X-ray castrates.

A group of 45 cases was studied, consisting of 24 surgical castrates, 11 X-ray castrates and 10 natural menopause. Vaginal smears were taken daily by the patients themselves with small glass

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¹ Papanicolaou, G. N., and Shorr, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 585.

² Papanicolaou, G. N., *Am. J. Anat.*, 1933, **52**, Supplement.

³ Frank, R. T., Salmon, U. J., and Friedman, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1666.

⁴ Frank, R. T., and Salmon, U. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 311.

pipettes. The smears were fixed in methyl alcohol and stained with aqueous fuchsin. Preliminary smears were taken for a period of 5-21 days. Gonadotropic hormone (G.H.) determinations were performed on the urine of 22 cases according to the technique recently reported from this laboratory.³ After a preliminary period of study of both the vaginal smears and G.H. excretion, 20 of these patients were given a concentrated oily preparation of estrogenic substance intramuscularly (Progynon-B—the benzoic acid ester of dihydro-folliculin).† The estrogenic preparation employed contains 2,000 rat units per cubic centimeter of oil. The dosage used in these cases varied from 4,000 R.U. to 36,000 R.U. given in divided doses of 4,000 R.U. at 3-4-day intervals.

We employed the following criteria for the classification of vaginal smears.

I—Preponderance of leucocytes associated with a large number of oval shaped epithelial cells containing large nuclei (“compact cells”).

II—Predominance of squamous epithelial cells; variable number of compact cells and leucocytes.

III—Squamous epithelial cells in large numbers; few leucocytes; total absence of compact cells.

IV—Large squamous epithelial cells with clear staining small nuclei; no leucocytes; no compact cells.

A I or II reading was interpreted as a negative phase; a III or IV reading as a positive phase.

During the preliminary period of observation we found consistently negative smears, (that is the I or II readings), in 18 of the 45 cases. In 14 cases the smears varied from I to III readings during the preliminary period of observation. Persistently positive smears (III and IV readings) were found in 13 cases (7 surgical, 2 X-ray castrates and 4 spontaneous menopause).

G.H. was found in the urine of the 22 cases in which the determinations were made. The G.H. excretion varied from 5 to 100 R.U. per day. There was apparently no relationship between the type of vaginal smear and the quantity of G.H. excreted.

In regard to the symptoms, we found no correspondence between the type of smear and the number or intensity of the flushes. Four of the cases that had the most severe menopause symptoms had consistently positive smears during the preliminary period, whereas 4 cases with typical negative smears had no flushes, whatsoever.

† This material was obtained through the kindness of Dr. Gregory Stragnell of the Schering Corporation, Bloomfield, N. J.

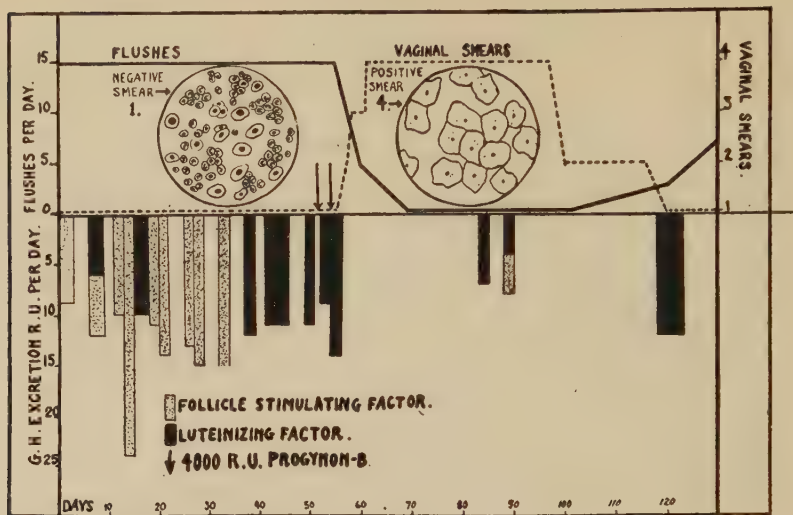


FIG. 1.

Effect of estrogenic substance (Progynon-B) upon vaginal smear, G.H. excretion, and symptoms of a surgical castrate.

Patient—age 50—three years after hysterectomy and bilateral salpingo-oophorectomy. Moderately severe symptoms, 15 flushes daily.

Following the administration of progynon-B, the smears of those cases which had been negative, changed to a IV within 5 days. This maximum effect lasted for about 4 weeks. Return to the preliminary negative phase occurred gradually during the course of 6-10 weeks after treatment. In cases with preliminary III smears, after the Progynon-B injection, these changed to IV.

Amelioration of symptoms coincided with the change to the IV smear. Gradual recurrence of symptoms ran parallel with regressive change in the smear to the preliminary phase.

From these studies it appears that in only about 40% of castrates and post-menopause cases do we find uniformly negative smears. In about 30% of the cases, variations from a negative to a positive phase occurred. In the remaining 30% of the cases persistently positive smears are found even if taken daily over a period of 2 weeks.

Whereas in untreated cases there does not appear to be any correspondence between the severity of the symptoms and the character of the smear, nevertheless, following the administration of adequate amounts of estrogenic substance, striking changes occur in the vaginal smear concomitant with the amelioration of symptoms. Recurrence of symptoms ran parallel with the gradual return of the smear to its preliminary status.

8471 P

Estrogenic Substances in the Blood and Urine After Castration and the Menopause.

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We noted that the blood cycle persists after hysterectomy.¹ We reported the recovery of estrogenic substances from the blood² and urine³ years after the onset of the physiological menopause. The present report deals mainly with the finding of estrogenic factor in the urine of human castrates.

The report is based on the study of 12 female surgical castrates, 5 physiological menopause patients and 3 X-ray castrates.

1. All of the 12 surgical castrates excreted estrogenic factor in the urine. The amount varied between 15-200 M.U.L. In 2 cases, aged 23 and 44 years, studied over a month's period, a total of 255 M.U. and 720 M.U. respectively, were excreted. The average normal excretion of a menstruating woman in the same period equals 1500 M.U.³ Blood studies of these 2 cases failed to show estrogenic factor.

2. No correlation could be demonstrated between the relative amount of estrogenic and prepituitary gonadotropic factor of the urine. Seven patients were studied. With high readings of both luteinizing and follicle stimulating factor as little as 15 M.U.L. of estrogenic factor and as much as 200 M.U.L. were encountered.

3. No difference was noted between castration (2 cases) and castration with removal of the uterus (10 cases).

4. The subjective symptoms (flushes, arthritis) showed no correspondence with the amount of estrogenic factor excreted—6 "mild" cases with from 40 to 200 M.U.L.; 6 "severe" cases with 15 to 200 M.U.L.

5. The vaginal spreads⁴ showed no correlation with the amount

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¹ Frank, B. T., *The Female Sex Hormone*, p. 256, 1929, Charles C. Thomas, Springfield, Ill.

² Frank, R. T., *N. Y. State J. of Med.*, 1934, **34**, 1009.

³ Frank, R. T., Goldberger, M. A., and Spielman, F., *J. Am. Med. Assn.*, 1934, **103**, 393.

⁴ Salmon, U. J., and Frank, R. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **33**, 612.

of estrogenic substance recovered or the severity of the symptoms. Positive spreads occurred with as little as 15 M.U.L., negative with as much as 200 M.U.L.

Of the 5 physiological menopause patients (ages between 44 and 59 years, duration 1 to 9 years) 2 showed a positive estrogenic blood reaction with 40 cc. of blood; 3 excreted from 60 to 400 M.U. per month. In only 2 (1 of which gave a positive blood reaction) was the excretion nil. The subjective symptoms of all 5 patients were severe.

The 3 X-ray castrates corresponded to the surgical castrates in their hormone titres.

Summary. The estrogenic factor continues to be excreted after surgical removal of the ovaries, as well as after the physiological menopause and X-ray castration. In spite of the presence of the estrogenic factor, excessive production of the gonadotropic factor (luteinizing and follicle stimulating) takes place.

No explanation of the source of the estrogenic factor, after the removal of the ovaries, can as yet be offered. Experiments to solve this problem are under way.

8472 C

Development of Incompatibilities in Dogs by Repeated Infusions of Red Blood Cells.*

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In the course of an investigation on the influence of diet upon the regeneration of serum protein it was found, as has been reported by others,¹ that with repeated plasmaphereses the hematocrit values tended to fall to anemia levels unless red blood cells from donors were injected periodically. Inasmuch as a fraction of the reinjected cells breaks down and liberates hemoglobin, which serves in the

* The expenses of this investigation were defrayed by a grant from the Research Fund, Yale University School of Medicine. These data will form part of a dissertation to be presented by Daniel Melnick to the Graduate School, Yale University, for the degree of Doctor of Philosophy, June, 1936.

† Standard Brands Inc. Fellow, 1934-1936.

¹ Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 251.

same capacity as dietary protein, an attempt was made to determine the degree of hemolysis *in vivo* in order to evaluate the liberated globin in terms of protein intake. Wu's² ultra-micromethod for the determination of hemoglobin was adapted, as modified by Bing³ and McFarlane.⁴

In all of the 4 experiments performed, the determination of hemoglobin on the citrated plasma, saline washing, and the modified Locke's solution⁵ in which the red cells were suspended for reinjection, indicated a remarkable uniformity in the hemolytic effects of these preliminary manipulations. The initial infusions of cells from the same donor (A) into 2 dogs (1 and 2) produced a proportional but negligible hemolysis. However, when cells from this donor (A) were injected a *second* time approximately 2 weeks later, a marked hemolysis *in vivo* was observed which failed to be related proportionally to the volume of cells injected.

TABLE I.

	—Dog 1—		—Dog 2—	
Experiment No.	I	II	III	IV
Cell volume injected, cc.	20	88	66	60
Maximum hemolysis <i>in vivo</i> , mg. %	5.8	187	15.5	52

Thus, in dog 1, the hemolysis, over and above a hemoglobinuria, was more than 30 times that recorded after the first cell infusion, although the cell volume injected was only $4\frac{1}{2}$ times greater. In the case of dog 2, where similar cell volumes were injected, the maximum hemolysis was found to be more than 3 times greater subsequent to the second transfusion.

For the evaluation of proteins with respect to their ability to stimulate serum protein regeneration, 2 larger dogs (No. 3 and No. 4) were used. During the course of repeated plasmaphereses upon these animals we noted that the initial injections of cells from donors A and B were tolerated very satisfactorily. This was interpreted to be proof of compatibility, although recipients and donors were not typed. Preliminary cross-agglutination tests *in vitro* had indicated perfect compatibility of donor A with dogs 1 and 2. According to the literature, different blood types in dogs have been infrequently observed and these have been interpreted as of no clinical impor-

² Wu, H., *J. Biochem.* (Japan), 1922, **2**, 189.

³ Bing, F. C., and Baker, R. W., *J. Biol. Chem.*, 1931, **92**, 589.

⁴ McFarlane, W. D., and McKenzie Hamilton, R. C., *Biochem. J.*, 1932, **26**, 1050.

⁵ Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **52**, 54.

tance.⁶ However, with repeated injections of the saline-washed red blood cells we have noted evidence of an incompatibility beginning with a mild hemoglobinuria, increasing in severity and resulting ultimately in profound shock. These infusions averaging 140 cc. of red blood cells in a total volume of 360 cc. were spaced from 7 to 10 days apart. In the interim the dogs were subjected to active plasmaphereses using homologous cells.

Ottenberg and Thalhimer⁷ have produced an analogous picture in their study on cats. By repeated transfusions immune isoagglutinins and isohemolysins were developed in the recipients. Subsequent transfusions resulted in no unfavorable reactions other than a hemoglobinuria.

The development of the incompatibility in our experimental animals suggests a common mechanism—a sensitization induced in the recipient by repeated injections of an antigen in the red blood cells of the donor.‡ The clinical picture of the reaction obtained in dogs 3 and 4 is characteristic of anaphylactic shock.⁸ There was intense salivation, vomiting, labored respiration, and involuntary urination and defecation while the injection was being made. The blood pressure was so very low that the femoral artery could hardly be palpated. The blood coagulability was so markedly reduced that hemorrhages from the sites of puncture in the femoral artery and jugular vein could be controlled only with great difficulty: tremendous hematomas were obtained in the thighs. Muscular weakness associated with a marked ataxia was common. Intensive hemoglobinuria was noted which continued until the animal's hematocrit approximated that recorded previous to the infusion. Indeed, dog 4 became markedly jaundiced, as evidenced by the intense yellow pigmentation of the cornea and skin.

The time between the collection and reinjection of the washed cells bore no relationship to the shock reaction; *i. e.*, homologous cells, even after being in the refrigerator 96 hours, were utilized satisfactorily, whereas heterologous cells, after a 3-hour period in the refrigerator, caused an immediate reaction.

The incompatibility was "one-sided" in that the sera of the recip-

⁶ Wiener, A. S., *Blood Groups and Blood Transfusion*, Springfield, Ill., Charles C. Thomas, 1935.

⁷ Ottenberg, R., and Thalhimer, W., *J. Med. Res.*, 1915, **3**, 213.

‡ We are greatly indebted to Dr. G. H. Smith, of the Department of Immunology, for suggesting a working hypothesis, and to Dr. W. M. Hale for his many helpful suggestions.

⁸ Topley, W. W. C., *An Outline of Immunity*, Baltimore, Md., William Wood and Co., 1933, pp. 194-195.

ients agglutinated the cells of the donors, whereas cells of the recipients were compatible with the sera of the donors.

The question arose whether the recipients were sensitized specifically against the present donors, against all dogs or against certain dogs, the cells of which have an antigenic make-up similar to that of the original donors. To investigate this, we carried out micro-agglutination tests *in vitro*, employing the cells of 25 dogs chosen at random and the sera of dogs 1, 3 and 4. The following conclusions seem warranted:

1. The sensitized dogs were incompatible with 50% of the dogs tested, although none of the entire group tested had ever served as donors. The suggested explanation is that these non-compatible animals have an antigenic make-up in their red blood cells similar to that of the original donors. Successful transfusions into the sensitized dogs were subsequently made with the cells from those animals that were shown to be compatible by *in vitro* tests.

2. The compatibility of the cells of any given dog with the sera of our recipients is independent of breed or sex.

3. Dogs 3 and 4, having been sensitized against the cells of both donors A and B, reacted in exactly the same manner to the cells of all animals tested. In the case of dog 1, which had been sensitized only against the cells of dog A, we also obtained a similar response. Apparently both dogs A and B have in their red cells a common antigen, which is possessed also by approximately 50% of the dogs.

4. Further evidence for initial compatibility was observed when dogs representative of each of the 2 groups were cross-typed.

A passive transfer test was made employing a dog characteristic of that group still compatible with the sensitized dogs. This animal, having never been the recipient of cells from the original donors, was shown by tests to be compatible with them. Plasma to the extent of approximately one-fourth of the animal's blood volume was injected, citrate being used as the anticoagulant. On the following day the animal evidenced *in vitro* an incompatibility with the donors. Intravenous injection of a cell suspension from donor A produced a syndrome suggesting mild anaphylaxis. Although we failed to observe the clinical symptoms of shock, the animal showed a marked nystagmus which persisted 2-3 minutes. The normal coagulation time of this dog's blood was 2 minutes; after the injection of the cells the coagulability was so markedly decreased that the blood failed to clot even after 6 hours. A test made 18 hours later showed normal coagulation time. The per cent

eosinophiles did not vary but remained at the normal level of 2-3%.§ With the next urination a marked hemoglobinuria was noted which persisted for more than 48 hours.

Another dog, belonging to that group which was incompatible with the sensitized dogs, received an injection of plasma (citrated) which was approximately one-fifth of its blood volume. This injection was alone sufficient to cause an intense hemolysis *in vivo* with a corresponding hemoglobinuria. This harmonizes with the observation of hemolysis in our recipients (dogs 3 and 4). The hemolysin which developed in these animals acted to destroy all of the donor's cells. By reversing the situation, *i. e.*, by injecting the sensitized serum into the blood stream of an incompatible dog, all of the cells of which were "foreign" to the hemolysin, an immediate and continuous hemolysis took place. Thus, whereas the normal hematocrits of this dog averaged 48%, the hematocrit on the day subsequent to the injection was 22%. The cell volume for the next 2 days approximated the same low value and was associated with an intense hemoglobinuria. The animal was beginning to show the characteristic yellow pigmentation of jaundice. The dog was found dead on the following day. Necropsy did not reveal any abnormalities other than paleness of the visceral organs characteristic of anemia.

In the course of our investigations we have noted repeatedly that the compatibilities between the recipients and donors were restored within 5-10 weeks after the last infusion of cells. These were evidenced both by agglutination tests *in vitro* and the performance of successful infusions of cells obtained from these donors. However, within the following week agglutinations *in vitro* were observed when cell suspensions of the donors were added to the sera of the recipients. These phenomena are truly characteristic of anaphylaxis.⁹

Five dogs of the compatible group have been subsequently employed as blood donors, enabling us to continue our original investigation. The sensitized dogs have received from these donors a total of 28 infusions of red blood cells spaced for the most part 7-14 days apart. No evidence of incompatibility was observed subsequent to these injections, nor have we noted the appearance of any agglutinins for the cells from these donors. The non-development of

§ Thanks are due to Dr. J. M. Orten for the determinations of the coagulation time of the blood and for the differential white cell counts.

⁹ Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology with a Section on Pathogenic Protozoa*, New York, N. Y., D. Appleton-Century Co., 1935, p. 258.

antibodies for these cells after the numerous infusions is indicative that the present donors will remain compatible with the sensitized dogs.

Inasmuch as dogs, for all practical purposes, are of the same group, so that any dog may be a suitable donor for another, the development of the incompatibilities in this species bears a striking similarity to the clinical picture obtained in some cases with humans. Wiener⁶ states that "in cases where the same patient is to be given more than one transfusion, the cross match should be repeated before each transfusion, even if the same donor is used again. Several cases have been reported in which, although the first transfusion was successful, reactions took place during subsequent transfusions where the same donor was used. . . . This subject has not been investigated thoroughly and deserves further study."

8473 C

Comparative Minimal Hypnotic Effects, Toxicity, and Pathology, Produced by Sodium and Magnesium Salts of Phenobarbital.

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College of Medicine, Dallas, Texas.*

Gilman and Barbour¹ have shown that the minimal hypnotic dose (M.H.D.) and the minimal lethal dose (M.L.D.) of magnesium phenobarbital when administered orally to rats are approximately 25 mg./kilo and 275 mg./kilo, respectively. According to the criterion of Issekutz² this would suggest a "hypnotic range" of 250 mg./kilo in the rat, or 10 times the M.H.D.

In a series of 232 rats we have found that when sodium phenobarbital and magnesium phenobarbital in freshly made 4% solutions are administered subcutaneously to rats in parallel experiments that there is no essential difference in the toxicity of either salt. The M.L.D. of both salts for rats by the subcutaneous route is close to 215 mg./kilo.

In dogs, the M.L.D. of both the sodium and the magnesium salts

¹ Gilman, A., and Barbour, H. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1634.

² Issekutz, B., *Therap. Monatshefte*, 1915, **29**, 379.

of phenobarbital, when administered orally, is in the neighborhood of 115 mg./kilo, and the M.H.D. of each salt is approximately 20 mg./kilo. However, in a series of 28 dogs a comparison was made of the minimal hypnotic response to the intravenous administration of both salts. By the intravenous route we found the M.H.D. to be 15 mg./kilo. No difference in degree of hypnosis was observed in dogs administered less than 30 mg./kilo, but at this dose level magnesium phenobarbital produced a more profound hypnosis than sodium phenobarbital. The dogs administered sodium phenobarbital would nod, or sleep lightly after onset of hypnosis, but were sensitive to noises and would come when called. On the other hand, the dogs administered magnesium phenobarbital would sleep soundly when left alone, and would neither come when called nor respond to noises of the same pitch and intensity as the dogs administered sodium phenobarbital. Further, the excitement state, manifested by struggle, muscular tremors, and rigidity appeared to be somewhat more depressed in the latter than in the former. The depressing effect of the magnesium ion as opposed to the stimulating effect of the sodium ion, when administered intravenously, may be a factor.

Twenty-eight dogs were used in comparative chronic toxicity experiments extending over a period of 60 days. Fourteen dogs of the group were administered daily doses of sodium phenobarbital by stomach tube and 14 dogs of the group were administered daily doses of magnesium phenobarbital in like manner. Details are shown in Table I. The tissues of dogs dying from chronic poisoning before the end of the 60-day period were examined histologically. One dog of each series that survived was sacrificed at the end of the period for histological studies. Although diffuse and diversified tissue changes were frequently observed in the tissues of dogs dying before the end of the experiment, all of the tissue changes observed could be explained on the basis of a profound vascular disturbance, and no constantly occurring lesion which might be characteristic of chronic poisoning by either the sodium or the magnesium salt of phenobarbital could be established in the nervous system or elsewhere.

Following the chronic toxicity experiments outlined in Table I, 7 dogs were administered orally by stomach tube 25 mg./kilo of sodium phenobarbital daily for 30 days, and 7 dogs were likewise administered 25 mg./kilo of magnesium phenobarbital for the same length of time. All dogs survived the 30-day period without constant weight loss or weight gain. Toward the end of the experiment it was evident that a marked degree of tolerance in both groups of

TABLE I.
Comparative Toxicity of Sodium Phenobarbital and Magnesium Phenobarbital
when Administered by Stomach Tube in 4% Solutions.

Dose Sodium Phenobarbital mg./kilo	No. Dogs	Deaths
10	2	1 death in 28 days; 1 death in 30 days.
15	3	1 " " 26 " 2 dogs survived.
20	3	All dogs survived.
25	2	" " " "
35	2	1 death in 5 days; 1 death in 10 days.
50	2	1 " " 3 " 1 " " 4 "
Dose Magnesium Phenobarbital mg./kilo		
10	2	All dogs survived.
15	3	1 death in 18 days; 2 dogs survived.
20	3	1 " " 16 " 2 " "
25	2	All dogs survived.
35	2	1 death in 4 days; 1 dog survived.
50	2	2 deaths in 3 days.

dogs had developed. At the end of the 30-day period 2 dogs of each group were taken off the drugs in order to study withdrawal symptoms. The withdrawal symptoms were essentially as described by Seevers and Tatum³ in chronic experimental barbitol poisoning, but the 4 dogs made a complete clinical recovery. The daily doses of the remaining dogs, 5 dogs of the sodium phenobarbital series and 5 dogs of the magnesium phenobarbital series, were raised to 35 mg./kilo. After 4 days the daily doses of each series were increased to 50 mg./kilo, which was survived by all dogs in both series for 14 days. In Table I it is shown that none of the 4 dogs survived the initial 50 mg./kilo dose for over 4 days. None of the dogs survived in either group when an attempt was made to build up the tolerance of the animals beyond the latter dose level.

Conclusions. There is no essential difference in the toxicity of the sodium and magnesium salts of phenobarbital when administered orally to rats and dogs. When magnesium phenobarbital is administered intravenously to dogs in doses of 30 mg./kilo it produces a more pronounced hypnotic effect than sodium phenobarbital likewise administered at the same dose level. It is apparent that the additive depressing effect of the magnesium ion, as opposed to the stimulating effect of the sodium ion, is responsible for the difference in degree of hypnosis of the 2 salts. No such effect was observed when the 2 salts of phenobarbital were administered orally.

³ Seevers, M. H., and Tatum, A. L., *J. Pharmacol. and Exp. Therap.*, 1931, **42**, 217.

In a final paper it will be shown that while the nervous system and other tissues of dogs dying from chronic phenobarbital poisoning contains no constantly occurring lesion typical of phenobarbital poisoning, phenobarbital produces a profound vascular disturbance in those tissues. In dogs a marked degree of tolerance to phenobarbital may be built up. After a period of pronounced irritability and sleeplessness, following withdrawal of the drug, clinical recovery takes place. The question of whether histological recovery parallels clinical recovery remains to be studied.

8474 C

Inoculation of Mice with Poliomyelitis Virus.

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Nungester¹ demonstrated that when poliomyelitis virus was combined with mucin and injected intraperitoneally into 76 mice, death occurred in 22, muscular weakness in 13 and flaccid paralysis in 2 animals. The brains and cords of 12 of the 22 mice that died and 7 controls were examined by Weil.² In 2, the picture seen closely resembled the histopathology found in *M. rhesus* monkeys that died of poliomyelitis. These results were sufficiently important to encourage further work along the same line.

Commercial gastric mucin powder was sterilized dry by autoclaving, a 5% emulsion prepared and used as a diluent to make up a 2% suspension of potent poliomyelitis virus. All injections were made the same day that the mixture was prepared and each mouse received 1 cc. intraperitoneally. The results are tabulated in Table I. In controls, the diluent was either normal saline, normal cord in mucin, or mucin in saline. In one group, the diluent for the mucin and virus was convalescent poliomyelitis serum.

All mice of group I that died following the inoculation of virus and mucin showed a small amount of clear, straw-colored fluid in the peritoneal cavity. The upper part of the small intestine was swollen and yellow reddish brown in color. There was slight or

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¹ Nungester, W. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1128.

² Weil, W., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1242.

TABLE I.

Group	Injection Material	No. of Mice	Deaths	% of Deaths
I	2% virus in 5% mucin	57	23	40.+
II	2% " " normal saline	30	1	3
III	2% normal cord in 5% mucin	30	4	13
IV	5% mucin in normal saline	15	0	0
V	2% virus, 5% mucin and convalescent serum	30	5	17

moderate hyperemia of the lungs. A few had enlargement of the spleen and hyperemia of the liver. Autopsy examination of the mice of groups II and III showed their organs to be normal, while those of group V showed some swelling and redness of the small intestine, but it was distinctly less marked than that seen in the animals of group I.

The spinal cords, lungs and livers of all animals that died were examined microscopically. The lung and liver sections were stained with hematoxylin and eosin, and the cord by Nissl's stain.

Cords. Sections of the cords of 11 animals of group I showed some round cell infiltration and neuroglial increase throughout the gray matter. The capillaries were noticeable, but none of the sections showed perivascular cuffing. The anterior horn cells were absent in many specimens and where they were present, both nucleus and cytoplasm showed disintegrative changes. No hemorrhages were noted. In sections of the cords of 2 animals that died in group III, the findings were the same as in group I. One specimen was normal. Sections of 3 of the cords of those dying in group V showed round cell infiltration that was almost as severe as that of group I.

Lungs. Microscopic examination of the lungs of those dying in group I showed generalized capillary dilatation and engorgement, which encroached upon the alveolar spaces without any fixed tissue proliferation. The bronchial mucosa was normal and there was no exudate. Pneumonia was found in the lungs of 2 of the 4 animals that died in group III. A slight hyperemia was found in the lungs of 3 animals of group V.

Liver. The animals that died in group I had intense capillary dilatation and engorgement of the central veins with focal collections of round cells in the parenchyma and in the interlobular spaces, especially around the bile ducts. The cytoplasm of the liver cells showed diffuse granular degeneration, indistinct cell boundaries, and a shrinkage of the nuclei with occasional fragmentation. In a few sections, lobular degeneration had occurred and had gone on to necrosis. These reactions, however, were seen in sections of the

liver from animals of other groups, though comparatively less in extent.

Twelve animals were sacrificed to obtain normal sections. Four of the 12 supposedly normal liver sections had focal collections of round cells in the perilobular position, although they were comparatively less extensive than those seen in sections of those animals that died after injections of the mucin and virus mixture. Sections of other organs were negative.

Poliomyelitis virus in combination with gastric mucin caused death of less than half of the animals injected; the combination was 3 times more lethal than normal cord in mucin and 13 times more lethal than virus in saline.

Thirty animals were protected with human convalescent poliomyelitis serum. Three specimens of convalescent serum were tried, each sample being tested in 10 animals. Complete protection was had by the 20 animals protected with the first 2 specimens. The third specimen may not have contained neutralizing antibodies, since death occurred in 5 of the 10 mice protected with this serum. Unfortunately all the serum of the third specimen had been used and the point as to whether or not there were any poliomyelitis neutralizing antibodies present could not be settled.

Since only 40+ % of the mucin and virus injected animals died, it is quite clear that mice cannot be used to test poliomyelitis virus where a 100% response is necessary. Our results might be changed by varying dosages to get a proper M.L.D. and further work would have been done along this line but for the fact that normal cord and mucin gave a comparable picture in 13% of the animals and for the fact that subsequent injections of another group of 20 animals with the same virus percentage from another lot of virus caused the death of only 4 animals. None of the controls of the latter group died.

Ten monkeys were injected intracerebrally with varying percentages and amounts of single and combined mouse cord suspensions, but the disease was not produced.

We confirm the observations of Nungester¹ that mucin added to poliomyelitis virus sometimes accentuated its potency when injected into the mouse. It is clear, however, that this animal cannot be used for experiments with poliomyelitis virus because of the inconstant results. The pathological sections examined by us were not distinctive enough to be considered typical. We were unable to infect monkeys with the cord emulsions of the mice that died.

8475 C

Antipyretic Action in Rats of Tolysin Alone and in Combination with Phenacetin.

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The relative therapeutic effectiveness of tolysin has been questioned because of the fact that large quantities can be tolerated without toxic effects. This has sometimes been attributed to inefficient absorption. Sufficient evidence has now accumulated to show that tolysin is absorbed in full therapeutic doses in humans, dogs and rats.¹⁻⁴ To date, however, the margin existing between therapeutic dosage and the limit of absorption has never been clearly demonstrated. Previous investigation has shown that 1,000 Mg./K. of tolysin, when given to rats daily by oral administration was completely absorbed and non-toxic, as evidenced by normal growth rate, absence of symptoms or autopsy findings.⁴ The therapeutic response to 25% of this dose is depicted in the following:

Antipyresis in rats was used as the basis for the evaluation of drug action. The animals were febrile by the subcutaneous injection of yeast, using the technique described by Smith and Hambourger.⁵ This results in a fever maintained at a fairly constant high level for over 30 hours. Such a lasting fever is a necessity in the study of antipyretics characterized by delayed onset and long duration of action.

During experimentation, the rats were kept in a constant temperature room at 86°F. Drugs were administered by stomach tube, in an acacia suspension, 18 hours after the removal of food. A single experiment consisted in temperature measurements not only on 4 rats for each drug given, but also on 4 fever controls, and 4 room controls. The antipyretic effects recorded are corrected for variations noted in the 2 control groups.

The antipyretic effect of tolysin on febrile rats receiving 250

¹ Barbour, H. G., Lozinsky, E., and Clements, C., *Am. J. Med. Sci.*, 1923, **165**, 708.

² Fuerth, O., and Kuh, E., *J. Pharmacol. and Exp. Therap.*, 1930, **38**, 57.

³ Barbour, H. G., and Lozinsky, E., *J. Lab. and Clin. Med.*, 1923, **8**, 217.

⁴ Barbour, H. G., and Gilman, A., *J. Pharmacol. and Exp. Therap.*, 1935, **55**, 400.

⁵ Smith, P. K., and Hambourger, W. E., *J. Pharmacol. and Exp. Therap.*, 1935, **54**, 346.

Mg./K. of the drug is depicted in Fig. 1. This action differs from that of most analgesic-antipyretic drugs in that the onset of action is delayed for 2 hours, whereas its duration is prolonged for over 8 hours. From the 2°F . temperature fall noted, it is obvious that $\frac{1}{4}$ of the amount of tolysin that can be easily absorbed by rats following a single oral administration has marked therapeutic action.

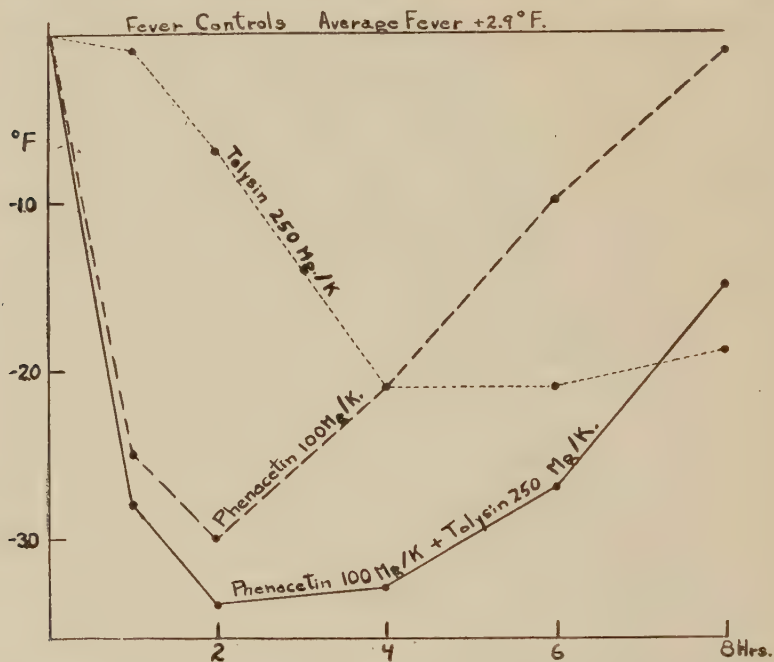


FIG. 1.

The antipyretic action of tolysin, phenacetin, and their combination.

The analgesic-antipyretic drugs commonly used are characterized by prompt action, but are handicapped by short duration of activity. In view of the nature of tolysin antipyresis as regards delayed onset and prolonged effect, combination with a quicker acting drug should result in rapid onset and prolonged duration of action.

Using the technique described above, phenacetin (100 Mg./K.) alone, or in combination with tolysin (250 Mg./K.) was given orally to fevered rats. Figure 1 shows the average results obtained on 56 animals, divided equally between phenacetin alone and the combination of phenacetin plus tolysin.

It can readily be seen that the maximum effect of phenacetin is obtained almost immediately and rapidly wanes after 2 hours. The antipyresis from tolysin is obtained more slowly and tends to be

more lasting. The most pronounced effect from tolysin is not observed until 4 hours after administration and continues practically unabated for 4 more hours. The results obtained by combined administration, as would be expected, are additive. Maximal effects are obtained at a time when both drugs are active. However, effective tolysin antipyresis persists after that of phenacetin, giving a sustained and powerful action.

TABLE I.
Toxicity of Phenacetin, Tolysin, and Phenacetin and Tolysin.

Drug	Dose G./K.	No. Rats	No. Survivals		% Survival	
			24 Hr.	48 Hr.	24 Hr.	48 Hr.
Tolysin	15	8	8	8	100	100
Phenacetin	6	8	0	0	0	0
Tolysin	15	8	5	4	67½	50
+	+					
Phenacetin	6					
Phenacetin	4	15	2	1	13	7
Tolysin	10					
+	+					
Phenacetin	4	15	8	2	57	13
Phenacetin	2					
Tolysin	5					
+	+	15	15	11	100	73
Phenacetin	2					

In a combination of drugs, it is necessary to determine to what extent the toxicity of the more toxic component may be enhanced by the other drug. In the present instance both drugs are relatively benign. As can be seen from Table I, tolysin is non-toxic to rats in doses as high as 15 G./Kilo, probably because of the limits of absorption. Phenacetin, the more toxic element of the combination, when administered orally has a minimal lethal dose (50% fatality) between 2 and 4 G./K. Combinations of tolysin and phenacetin in a ratio of 2½:1, are no more toxic per given amount of phenacetin than phenacetin alone. When the amount of phenacetin in such a combination exceeds the m.l.d., the presence of so much tolysin even proves protective. This protection is probably the result of a mechanical effect in the gut, large amounts of tolysin preventing the rapid absorption of phenacetin. When given in small doses, however, as shown above, the presence of tolysin in no way interferes with the absorption and antipyretic action of phenacetin.

1. Tolysin in doses of 250 Mg./K. exhibits in fevered rats a marked antipyretic action of slow onset, persisting for more than 8 hours. This amount represents less than 25% of the easily absorbable dose previously found by us.⁸

2. When this dose of tolysin is combined with phenacetin (100

Mg./K.) antipyresis of both rapid onset and long duration is obtained.

3. The presence of tolysin in no way augments the toxicity of phenacetin in rats.

8476 C

Permeability of Red Cell Membrane after Hypotonic Hemolysis.

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The generally accepted explanation for hypotonic hemolysis is that the red cells take up water, swell, and finally become permeable because their membranes are stretched or under tension. Conductivity measurements, however, show that the resistance of a hemolysed suspension is such that the cell membranes must have a very low conductance (Fricke and Curtis¹), an observation which forces one to consider the possibility that the increased permeability associated with lysis is temporary only, and followed by a more or less complete repair of the membrane.

1. The simplest evidence is to be obtained from systems in which "reversible hemolysis" occurs. This phenomenon is observed when several volumes of water are added to blood or washed cells so as to produce more or less complete lysis, and enough hypertonic NaCl to restore the original tonicity added subsequently. The opacity which appears on the restoration of the tonicity was thought by Brinkman and Szent-Gyorgi² to be due to the taking up of liberated hemoglobin on or in the cells, but Bayliss³ showed that it is the result of two occurrences: (a) if the amount of added water is small, swollen but unhemolysed cells shrink to their original size on the re-establishment of the tonicity, and (b), if the amount of added water is great, hemolysed ghosts shrink under the same conditions, and, trapping hemoglobin, bring about optical heterogeneity.

If to 10 cc. of a washed rabbit red cell suspension with a volume concentration of about 0.7 is added 40 cc. of water, a tonicity in the neighborhood of 0.2 is established, and this is low enough to hemolyse virtually all the cells, as can be shown by the hemoglobin con-

¹ Fricke, H., and Curtis, H. J., *J. Gen. Physiol.*, 1935, **18**, 821.

² Brinkman, R., and Szent-Gyorgi, A., *J. Physiol.*, 1923, **58**, 204.

³ Bayliss, L. E., *J. Physiol.*, 1924, **59**, 48.

centration in the supernatant fluid after "reversal" has been produced and the ghosts centrifuged off. One can then calculate the volume which the ghosts ought finally to occupy if they shrink, on the addition of the NaCl which brings about the "reversal", in the same way as the original cells swelled on the addition of the water, *i. e.*, as osmometers of the same degree of perfection. In Table I ρ_1 is the volume concentration of the cells before the addition of n volumes of water, ρ_2 the volume concentration expected if the hemolysed ghosts were to shrink to the volume of the original cells, and ρ_3 the volume concentration of the ghosts as found by spinning the system showing "reversal" in a high speed hematocrite (12,000 r.p.m. to constant volume, which is usually attained within an hour).

TABLE I.

ρ_1	n	ρ_2	ρ_3
0.68	4	0.113	0.105
0.43	4	0.072	0.070
0.80	5	0.114	0.120
0.84	5	0.120	0.115

Allowing for some uncertainty both as regards the calculated tonicities and the hematocrite determinations, the calculated and observed results agree closely. The simplest way of accounting for the return of the ghosts to the original volume occupied by the cells from which they were derived is that the permeability to NaCl of the membrane surrounding the ghosts is very small compared to the permeability to water. Yet at the moment of lysis the state of affairs must have been very different, for a red cell loses pigment into a hypotonic solution which hemolyses it as if the permeability of the membrane to hemoglobin were about $4(10^{-4})$ cm./sec., or as if the membrane had no less than 6 holes of 1μ diameter punched in it (Ponder and Marsland⁴); a membrane so permeable to hemoglobin must be allowed to have had a still greater permeability to NaCl, unless our ideas about red cell permeability and hemolysis are entirely wrong.

2. A concentrated suspension of ghosts can be made by hemolysing one volume of washed red cells with 4 volumes of water, adding one volume of NaCl hypertonic enough to restore the original tonicity (about 5% NaCl), spinning down the ghosts, and finally suspending them in a small amount of isotonic NaCl. The ghosts always contain a small amount of pigment, and it is impossible to remove this more than partially by washing. If a quan-

⁴ Ponder, E., and Marsland, D., *J. Gen. Physiol.*, 1935, **19**, 35.

tity of such a ghost suspension, of known volume concentration, is added to hypotonic NaCl and the volume concentration again measured (high speed hematocrite), it will be found that the ghosts swell in hypotonic solutions in very much the same way as do intact red cells, *i. e.*, as "imperfect osmometers" with R-values between 0.5 and 0.8. Again, in fact, they behave as if their membranes were much more freely permeable to water than to NaCl, and as if the highly permeable state associated with hemolysis had been followed by a "repair" of the membrane and a restoration of much of the original impermeability to cations.

3. Fricke and Curtis¹ could not independently determine the volume concentration of the cells in their hemolysed systems, and so were unable to say how completely non-conducting the cells were after hemolysis. In the case of suspensions of ghosts obtained by producing "reversal" of hemolysis, one can measure the volume concentration (a) by hematocrite, and (b) by conductivity, and from the correspondence between the 2 figures can form an estimate of how completely non-conducting the cells are. The volume concentration found by conductivity is obtained from the expression

$$\rho = (r_1/r_2 - 1)/(r_1/r_2 + 1/X)$$

in which r_1 is the resistance of the suspension of ghosts, r_2 that of the fluid in which the ghosts are suspended, and X a "form factor" which can vary between about 1.0 for wholly non-conducting rabbit red cells in their normal discoidal form, and 2.00 for wholly non-conducting spheres. Since the ghosts are crenated objects, there is doubt as to the real value of X , but Table II shows ρ_1 , the volume concentration found by hematocrite, ρ_2 , that found by conductivity with a value of 1.20 for X , and ρ_3 , that found by conductivity with a value of 2.00 for X .

TABLE II.

ρ_1	ρ_2	ρ_3
0.470	0.423	0.472
0.390	0.354	0.400
0.370	0.320	0.365
0.310	0.282	0.324
0.320	0.274	0.315

These figures show that excellent agreement as regards volume concentration would be reached if the ghosts could be considered as non-conducting spheres. Examination of the ghosts, however, shows them to be crenated, but essentially discoidal and certainly not spheres; there is, accordingly, a discrepancy between the results

for volume concentration obtained by hematocrite and the results found by conductivity on the assumption that the cells are altogether non-conducting. This discrepancy, however, is smaller than that which appears in the table between the values of ρ_1 and ρ_2 , and probably much smaller, both because the hematocrite tends to give incomplete packing and too large values for ρ_1 and because the form factor for the crenated ghosts is certainly not as small as 1.20. Suppose that the discrepancy in volume concentration really amounts to about 0.02 as a maximum figure after all experimental errors have been allowed for; calculation then shows that it would be accounted for if the ghosts had a specific resistance about 25 times that of the surrounding medium, instead of being completely non-conducting. As the interior of the ghost, like the interior of the intact cell, has a resistance about the same as that of the surrounding medium, and as the surface membrane is certainly very thin, this would mean that the specific resistance of the membrane is of the order of thousands of times that of the suspension fluid, *at least*. Such a result is in keeping with those obtained for the osmotic behavior of the ghosts, and, as it is very difficult to think of the membrane having such a high resistance at the moment of hemolysis, we are again led to the idea of a more or less complete "repair".

Summary. Both the osmotic and the electrical behavior of ghosts obtained by producing the so-called "reversal of hemolysis" in suspensions of red cells hemolysed with water lead to the conclusion that the membranes surrounding the ghosts have a high degree of semi-permeability. Existing evidence makes it very difficult to think of this high degree of semi-permeability prevailing at the instant of hemolysis, and so the idea that the red cell membrane becomes more or less "repaired" after lysis needs to be considered.

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